Genetic analyses of drought tolerance and resistance to late blight among potato genotypes

by

Hirut Getinet Betaw

MSc. Horticulture (Alemaya University, Ethiopia)

BSc. Plant Sciences (Alemaya University, Ethiopia)

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African Centre for Crop Improvement (ACCI)
School of Agriculture, Earth and Environmental Sciences
College of Agriculture, Engineering and Science
University of KwaZulu-Natal
Republic of South Africa

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Thesis summary

Potato is one of the valuable crops in Ethiopia serving as a source of food and income for smallholder farmers. About 70% of the country's arable land is suitable for potato production. Despite the rapid growth of the total potato production in the country, productivity of the crop under the small-scale farming sector is estimated at 11 t ha^{-1}, which is far below attainable yields of over 40 t ha^{-1}. Recurrent drought and late blight disease are the most important constraints affecting productivity of potato in Ethiopia. Late blight disease of potato, caused by *Phytophthora infestans,* is widespread in most potato growing areas of Ethiopia causing yield losses reaching up to 100% in susceptible cultivars. Breeding for host tolerance and resistance could be the best option for managing recurrent droughts and controlling the disease, respectively. The overall goal of this study was, therefore, to contribute to improved food security in Ethiopia by developing potato cultivars with improved yields, late blight resistance and drought tolerance. The specific objectives of the study were to (i) select late blight resistant and high yielding potato clones under field conditions in the north-western parts of Ethiopia, (ii) determine combining ability and gene action controlling late blight resistance, yield and yield components and to identify promising potato genotypes as potential parents in a breeding programme, (iii) determine combining ability and gene action controlling yield, yield components and drought tolerance related traits among selected potato clones and to identify promising parents and crosses for cultivar development, and (iv) assess the level of genetic diversity among 18 selected potato clones using 23 simple sequence repeat (SSR) markers and to complement phenotypic selection for identification of suitable parents for breeding. These objectives were achieved based on four sets of experiments conducted in the north-western Ethiopia.

Twenty-four selected potato clones, of which 17 from the B3C2 population acquired from the International Potato Centre (CIP) and seven widely grown released and farmers' cultivars, were evaluated for late blight resistance and yield related traits at three locations in the north-western Ethiopia. A randomized complete block design was used with two replications. Results indicated significant variation among the genotypes for late blight resistance and yield related traits across the three locations. The following five clones were selected: 396029.25, 395017.229, 396004.263, 396034.103 and 395077.12, displaying high to moderate resistance to late blight and greater yield levels.

Twelve parents selected from late blight resistant advanced population (B3C2) were crossed using North Carolina Design II. Eighteen F1 families derived from these crosses were evaluated at two late blight hotspot areas in Ethiopia. Combining ability and gene action were

determined for late blight resistance under natural epidemics. Results showed that the general combining ability (GCA) effect was more important than the specific combining ability (SCA) effect for the relative area under disease progress curve (rAUDPC), total tuber yield, marketable tuber yield and average tuber weight, indicating that the expression of these traits was controlled by additive gene action. The parents with good GCA effects for late blight resistance were: 396264.14, 395109.34 and 396004.263. The first two contributed towards high tuber yield. Crosses from 396004.263 x 395017.229, 395096.2 x 396012.288 and 395109.7 x 396264.14 were best specific combiners for late blight resistance.

Thirty-two potato families derived from crosses of two sets of 16 parents and 17 clones were field evaluated for yield and drought related traits in a 7 x7 lattice design with two replications under well-watered and managed drought stress conditions. Results revealed significant differences among genotypes for drought stress tolerance, growth, physiological and yield related traits. Significant GCA and SCA effects were detected among parents and crosses, respectively. The GCA effects were more important than the SCA effects for total tuber yield, marketable tuber yield, average tuber weight, plant height, chlorophyll content and groundcover, suggesting the predominance of additive over non-additive gene action for these traits under drought stress. The best general combiner parents for yield and drought tolerance were clones 395112.32, 396034.103 and 396012.288. The families with the best SCA effects for both tuber yield and drought tolerance were 395109.34 x 396041.102, 395096.2 x 396012.288, 395109.7 x 395017.14 and 396031.108 x 395017.14.

Eighteen selected clones phenotyped for drought tolerance and late blight resistance were genotyped using 23 polymorphic SSR markers to determine genetic distance and to select suitable parents for breeding. Results showed the presence of wider genetic diversity among the tested clones. Pair-wise estimates of genetic similarity ranged from 0.26 to 0.52 with the mean of 0.35. Ninety-five alleles were amplified and polymorphic alleles per locus ranged from 3 to 7 with a mean of 5. The mean polymorphic information content (PIC) values, observed heterozygosity and expected heterozygosity were 0.62, 0.78 and 0.68, respectively. The genotypes were clustered into three distinct groups. The following clones were selected from each cluster: 396029.25 from cluster I, 396038.107, 396038.101 and 395112.32 from cluster II, and 395017.229 and 395109.34 from cluster III, for drought tolerance and late blight resistance breeding.

In summary, the study demonstrated the existence of genetic variability among the tested clones for late blight resistance, drought tolerance and yield and related traits. The study identified promising potato genotypes with high combining ability for tuber yield, late blight resistance and drought tolerance. The selected parents and families will be further evaluated

for release in the highlands of north-western Ethiopia or similar environments in sub-Saharan African countries.

Declaration

I, Hirut Getinet Betaw, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original work.
2. The thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, picture, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
   a. Their words have been re-written but the general information attributed to them has been referenced.
   b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the reference section.

Signed

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Hirut Getinet Betaw

As the candidate's supervisors, we agree to the submission of the thesis:

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Prof. Shimelis Hussein (Supervisor)

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Dedication

This thesis is dedicated

To my son **Abel Asmamaw**

To my husband **Asmamaw Yeshanew** and my mother **Yayesh Dessie**

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Introduction to thesis

Background

Potato (*Solanum tuberosum* L., 2n=4x=48) is one of the most important food crops worldwide. It ranks third after rice and wheat in terms of human consumption (FAOSTAT, 2015). Potato contains significant amounts of vitamin C and essential amino acids, such as lysine useful in and complementing cereal based diets (Beukema and Van der Zaag, 1990; Hirpa et al., 2010). In comparison with cereals, potato produces more food energy and protein per hectare. Its high efficiency in producing energy and valuable protein, and its short vegetative period makes the potato a high potential food security crop. Potato is grown in about 149 countries, and consumed by more than a billion people worldwide (Birch et al., 2012). Developing countries produce about 30% of the world's potatoes, and the share of these countries in world production is growing rapidly (Agiro, 2011). The top ten African countries in terms of area allocated to potato production are Algeria, Egypt, Malawi, South Africa, Rwanda, Kenya, Morocco, Tanzania, Nigeria and Ethiopia (Table 0.1).

Table 0.1 The top potato producers in Africa in 2013

Countries	Harvested area (ha)	Yield (tons ha^{-1})	Production (tons)
Algeria	162,707	30.3	4,928,028
Egypt	178,000	27.0	4,800,000
Malawi	258,585	17.5	4,535,955
South Africa	66,000	34.1	2,252,000
Rwanda	164,691	13.6	2,240,715
Kenya	152,007	14.4	2,192,885
Morocco	53,047	36.4	1,928,606
Tanzania	203,165	8.7	1,767,536
Nigeria	264,000	4.5	1,200,000
Ethiopia*	69,999	11.1	775,503

Adapted from (FAOSTAT, 2015), the data doesn't include off season production

In Ethiopia, potato is a source of both food and income in the densely populated highlands. It is grown by about 1.8 million farmers and remains the most important among the root and tuber crops produced in the country. The total production in 2013/2014 main and offseason was 1,612,006.2 t on 179,159 ha (CSA, 2014). The annual per capita consumption is estimated at 13 kg. However, potato production is expanding steadily over time (Haverkort et al., 2012). FAOSTAT (2015) estimates show that the main season production has increased from 349,000 in 1993 to 775,503 t in 2013. Seventy per cent of the country's arable land, mainly in highland areas (≥1,500 m), is believed to be suitable for potato production. The

highlands are inhabited by 90% of Ethiopia's population making potato a key crop in ensuring national food security (FAO, 2009). Potato is grown in four major regions of the country: the central, the eastern, the north-western and the southern. North-western Ethiopia is the top potato producing area inhabited by 40% of the country's potato farmers (Hirpa et al., 2010). South Gondar, North Gondar, East Gojam, West Gojam and Agew Awi are the main production areas in this region.

Despite high potential production environments and marked growth, the national average potato yield in farmers field in Ethiopia is only 11.1 t ha^{-1}, which is lower than the experimental yields of over 38 t ha^{-1} (Woldegiorgis, 2013; FAOSTAT, 2015). The low yields are the result of a number of production constraints mainly involving abiotic and biotic stress factors. Among the biotic constraints late blight, bacterial wilt, virus diseases and potato tuber moth constitute the major threats to potato production, while the abiotic stresses include soil nutrient deficiency, frost, drought and erratic rainfall (Gildemacher et al., 2009; Berihun and Woldegiorgis, 2013; Kolech et al., 2015).

Potato late blight, caused by the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, is a major disease that can result in total failure of the crop. It occurs throughout the major potato production areas of Ethiopia. The disease damages leaves, stems and tubers. Yield losses of susceptible cultivars can range from 30 to 100% (Kassa and Beyene, 2001; Mohammed, 2014). Research showed that late blight in Ethiopia has the A1 mating type (US 1 clonal lineage), which reproduces asexually (Schiessendoppler and Molnar, 2002).

The most effective control methods of the disease are host resistance and applications of fungicides. However, fungicide use is unaffordable in Ethiopia and has negative consequences for the environment and human health. Farmers in Ethiopia practice early planting to avoid condition that favour late blight development, regardless of yield penalty associated with lack of supplemental irrigation in the country. During 2014 only 1.3% of the total cultivated land is under irrigation (Forbes et al., 2003; CSA, 2014). Therefore, the principal method of late blight management should be host resistance (Colon et al., 1995). Advanced resistant breeding populations and selected clones have been developed by the International Potato Centre (CIP) for a variety of agro-ecological zones similar to sub-Saharan Africa potato producing areas. These advanced clones can be used as a valuable source of genetic variation in breeding programmes. Among these clones, 'population B recombination cycle 3 (Pop B3)' is the new source for durable late blight resistance. Some of the clones derived from this population, such as CIP-393371.58 (cultivar '*Belete*') showed promising performance and was released in Ethiopia (CIP, 2012). However, the performance

of CIP candidate clones under moisture stressed environments needs further study because drought is one of the most important yield limiting factors in Ethiopia.

In Ethiopia, water shortage occurs due to rainfall variability both temporally and spatially (Mersha and Boken, 2005). Studies showed that the area with stable rainfall has decreased, while the area with highly variable rainfall has substantially increased (Mersha, 1999; Viste et al., 2013). As a result, drought recurrence cycle shortens over time and currently it occurs every two years in different parts of Ethiopia (Berhan et al., 2011). Moreover, due to climate change, areas affected by dry spells are expanding (Hiskias, 2011). Before the 1980s, drought was most protracted in the northern and eastern regions. However, the number of drought-affected areas has dramatically increased and now includes the most productive regions in west and south Ethiopia (Hiskias, 2011). Various climate models have indicated that drought episodes will become more frequent because of long-term effects of global warming (Anithakumari et al., 2011).

The total amount of annual rainfall in many locations of the country is high, but its distribution is highly erratic. Rainfall distribution during the growing period was much more variable than the seasonal total, resulting in a limited growing period (Simane et al., 1994; Gebrehiwot et al., 2011). Dry spell probability during the main season is particularly high at the end of the growing season. Simane et al. (1999) reported that altitude had a significant negative relation with temperature, but not with precipitation amount and distribution. A 10% decline in average rainfall in Ethiopia below the long-term national average results in a 4.4% reduction in national food production (von Braun, 1991). The impact of rainfall on crop production can be related to its total seasonal amount or its intra-seasonal distribution. In the extreme case of droughts, with very low total seasonal amounts, crop production suffers the most. But more subtle intra-seasonal variations in rainfall distribution during crop growing periods, without a change in total seasonal amount, can also cause substantial reductions in yields. This means that the number of rainy days during the growing period is as important, if not more, as that of the seasonal total. Generally, the effect of rainfall variability on crop production varies with types of crops cultivated, types and properties of soils and climatic conditions of a given area (Bewket, 2009).

In Ethiopia, potato production is primarily dependent on natural rainfall that frequently fails to meet the water demand of the crop. The potato is more sensitive to soil water conditions than most other crop species. At all stages of the growth, water stress reduces photosynthetic efficiency, but drought during the period of tuber initiation and bulking has the most drastic effect on tuber yield and quality (Vayda, 1994; Anithakumari et al., 2011). However, there are differences in the degree to which individual cultivars are affected by moisture stresses

(Steyn et al., 1998; Schittenhelm et al., 2006; Schafleitner et al., 2007). Therefore, there is a possibility to improve yield for drought prone areas through selection and breeding for high yield under water stress conditions.

Selection of potato clones based on the combining ability estimates helps to identify the most valuable parents and crosses, and to recommend a strategy for clonal improvement. The importance of both additive and non-additive gene action in inheritance of yield and yield components under moisture non-stressed conditions have been reported in different studies (Brown and Caligari, 1989; Maris, 1989; Ruiz de Galarreta et al., 2006). However, little work has been done under water stressed conditions. As regards to late blight, different studies have shown that the additive component of genetic variance was larger than the non-additive in inheritance of quantitative resistance (Landeo et al., 2001; Kumar et al., 2007; Haynes et al., 2008).

Estimation of genetic diversity helps in the choice of parental combinations of the greatest promise (Gaur et al., 1978). The use of parents of diverse genetic origin is considered important in hybridization programs to maximize heterozygosity and maintain high levels of variability in the progeny (Biswas et al., 2008). However, Hung et al. (2012) found that genetic distances among maize parents had no predictive value for progeny variation. Genetic diversity studies using molecular markers, which are independent of environmental influences and tissue type, provide greater resolution and may complement phenotypic measurements (Demeke et al., 1996). Among molecular techniques, polymerase chain reaction (PCR) based approaches are in greater demand because of their simplicity and requirement for only small quantities of sample DNA. Microsatellites or simple sequence repeat (SSR) markers are easy and simple to use, specific, highly polymorphous and reproducible.

Information on combining ability and genetic diversity of parents for yield and resistance for late blight, under moisture stress and in the presence of late blight, in that order, can be useful for breeding cultivars with both drought tolerance and late blight resistance. Incorporating these traits in the same genotypes ensures the development of cultivars with high and stable yield potential under fluctuating rainfall conditions. This is important in improving the productivity of potato in farmers' fields in agro-ecologies with erratic and unpredicted rainfall conditions.

Rational for research focus

Ethiopia is among the leading ten sub-Saharan Africa countries in terms of area cropped to potato (FAOSTAT, 2015). Potatoes are a source of both food and cash income in the

densely populated highlands of the country. However, the national average yield of the crop is less than 11 t ha^{-1} which is much less than the attainable yields (Hirpa et al., 2010). The gap between national average and attainable yield is mainly due to late blight disease and water stress. Most potato farming occurs under rain-fed conditions by small-scale farmers who cannot afford irrigation and fungicide applications. In most potato growing regions, precipitation is inadequate and irregular, and is often failing to meet the water demands of the potato crop. The frequency of this problem is likely to increase owing to global warming (IPCC, 2007). Even though potato is often considered a drought sensitive crop, research has shown that potato genotypes differ in drought tolerance (Steyn et al., 1998; Schittenhelm et al., 2006; Schafleitner et al., 2007). However, very limited, research has been done on breeding potato for water deficit environments in Ethiopia.

Under Ethiopian unpredictable rainfall conditions, late blight, which is the disease of wet environments, should also be considered while breeding for drought tolerance. The disease is most devastating throughout the major potato producing areas. Several improved varieties with reasonable resistance to late blight have been released to farmers, although a number of them have been abandoned, as the apparent resistance to late blight was not durable. Therefore, breeding and selection for genotypes combining both late blight resistance and drought tolerance would be important to improve productivity of the potato in drought prone areas.

Research objectives

The objectives of the study were:

- To select late blight resistant and high yielding potato clones under field conditions in the north-western parts of Ethiopia.
- To determine combining ability and gene action controlling late blight resistance, yield and yield components and to identify promising potato genotypes as potential parents in a breeding programme.
- To determine combining ability and gene action controlling yield, yield components and drought tolerance related traits among selected potato clones and to identify promising parents and crosses for cultivar development.
- to assess the level of genetic diversity among 18 selected potato clones using 23 simple sequence repeat (SSR) markers and to complement phenotypic selection for identification of suitable parents for breeding.

Outline of the thesis

This thesis consists of five distinct chapters in accordance with a number of activities related to the above mentioned objectives (Table 02). Chapters 2-5 are written as discrete research papers, each following the format of a stand-alone research paper (whether or not the chapter has already been published). This is the dominant thesis format adopted by the University of KwaZulu-Natal. Some overlap and unavoidable repetition of references and some introductory information between chapters may exist. The referencing system used in the chapters of this thesis is based on the Journal of Crop Science referencing style.

Table 0.2 Thesis outline

Chapter	Title
-	Introduction to thesis
1	Literature review
2	Response of potato clones to late blight disease, yield and yield related traits in north-western highlands of Ethiopia
3	Combining ability of selected potato clones for resistance to late blight disease, yield and yield components
4	Combining ability of selected potato clones for drought tolerance and yield components
5	Genetic diversity analysis of selected potato genotypes using SSR markers
-	An overview of the research findings

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CHAPTER 1. Literature Review

1.1 Introduction

This literature review presents important aspects of breeding potato for drought tolerance and late blight resistance, emphasising on basic principles in diversity, genetic analysis and breeding methods. It provides the current state of knowledge and advances made in understanding the crop, the pathogen of late blight disease and the effect of drought on yield and related traits. This is followed by summary of the importance of the crop and information on the casual organism, epidemiology and life cycle, and symptoms of late blight disease. Different control measures for the disease, with more emphasis on breeding for durable resistance are described with screening techniques for late blight resistance and drought tolerance. Finally, cultivar development strategies including broadening the present genetic base and selection methods are discussed. An attempt has been made to focus on literature related to Ethiopia whenever relevant and available.

1.2 Origin and global spread of potato

The potato (*Solanum tuberosum* L., 2n=4x=48) has its origin in the Andean mountains of South America. It was first cultivated in the Andes in the vicinity of Lake Titicaca near the border of Peru and Bolivia. Potatoes seem to have been domesticated at least 7000 years ago (Heřmanová et al., 2007). Later, the potato crop spread through the Andes and by the time of the Spanish conquest in the early sixteenth century, farmers were cultivating hundreds of varieties throughout the highland area of Bolivia, Chile, Colombia, Ecuador, and Peru (Horton, 1987).

Potatoes were introduced to Europe by Spanish sailors between the16th and 17th century. From Spain, the potato spread to the continental Europe. By 1600, potatoes reached Italy and Germany from Spain. It is believed to have reached most parts of the world through the colonial powers, rather than directly from South America (Horton, 1987). Potato was introduced to Ethiopia in 1858 by the German botanist Schimper. Since then, it becomes an important crop in many parts of the country (Tsegaw, 2005).

1.3 Taxonomy and diversity of potato

The potato belongs to *Solanaceae*, the family of about 90 genera and 2800 species. The genus *Solanum* consists of about 2000 species (Heřmanová et al., 2007). The more economically important section of the genus *Solanum*, *Petota*, has about 110 tuber bearing species, that include the cultivated potato and its wild relatives (Spooner, 2009). Most species within *Petota* section are very similar and they are able to exchange genes encoding

various traits (Jacobs et al., 2011; Spooner et al., 2014). Wild potatoes are widely distributed in the Americas from the southwestern United States to the southern cone of South America (Camadro, 2012; Spooner et al., 2014). Due to a large range of geographical and ecological adaptation, wild potato species have often developed strong resistance to biotic and abiotic stresses and they are widely used in potato improvement programs (Bradshaw, 2007).

According to Spooner et al. (2014) the cultivated potato can be grouped into four species: 1) *S. tuberosum*, with two cultivar groups: a) *Andigenum* group of upland Andean genotypes consisting of diploids, triploids and tetraploids, and b) the *Chilotanum* group of lowland tetraploid Chilean landraces, from which the modern cultivars arose, 2) *S. ajanhuiri* (diploid), 3) *S. juzepczukii* (triploid), and 4) *S. curtilobum* (pentaploid). The modern cultivars are the products of extensive breeding between different cultivar groups and wild species (Spooner et al., 2014).

1.4 Cytogenetics of potato

The number of ploidy levels of potato species, based on a haploid number of 12, ranges from diploid (2*n* = 24) to hexaploid (6*n* = 72), and includes triploids, tetraploids, and pentaploids. Diploids are the most common species (Larrosa et al., 2012). The common potato *S. tuberosum* is a tetraploid (2*n* = 4*x* = 48) with gametes of *n* = 24 (Heřmanová et al., 2007). Most diploid tuber bearing *Solanum* species have the S-locus gametophytic self-incompatibility system (Camadro et al., 2004; Weber et al., 2012). In contrast to the diploid potatoes, polyploid species (both wild and cultivated) are self-compatible. Self-compatibility in polyploids could be explained by fixed heterozygosity at the S-locus, that is the occurrence of two different alleles in pollen grains (Camadro et al., 2004; Larrosa et al., 2012).

1.5 Genetic basis of hybridization barriers

There are unique genetic factors that cause hybridization barrier among species of *Solanum* section *Petota*. These barriers include interspecific pollen-pistil incompatibility, nuclear-cytoplasmic male sterility, and failure of the endosperm during fertilization (Camadro et al., 2004; Bradshaw et al., 2006). However, gene flow among reproductively isolated species (sympatries) is not completely blocked since the barriers are not complete (Larrosa et al., 2012).

Interspecific pollen-pistil incompatibility is an important and common pre-zygotic hybridization barrier (Spooner et al., 2014). When individuals from cross-incompatible species crossed, pollen tube growth impedes either in the top, middle or bottom of the style (Spooner et al., 2014). The mechanism of this hybridization barrier is not known although a gene-for-gene interaction between stylar tissue and pollen has been proposed (Camadro et al., 2004;

Weber et al., 2012; Spooner et al., 2014). There are some diploid species that show unilateral incompatibility, a phenomenon in which self-compatible species can be crossed as female, but not as male, to self-incompatible species. It is also possible to find exceptional plants that do not exhibit unilateral incompatibility in self-incompatible × self-compatible interspecific crosses. Identification of such plants allows breeders to overcome the incompatibility crossing barriers (Spooner et al., 2014).

The second reproductive barrier, which is common in potatoes, is nuclear-cytoplasmic male sterility. Specific interactions between cytoplasmic and nuclear genes of interspecific hybrids commonly lead to male sterility (Camadro et al., 2004; Spooner et al., 2014). Male sterility in progenies derived from the crosses of various wild and cultivated species have been reported by several authors (Jansky, 2011; Larrosa et al., 2012; Weber et al., 2012). This kind of barrier can be incomplete due to segregation of genes involved in the incompatibility reactions in pollen and pistil (Larrosa et al., 2012). Segregation for male sterility has also been reported in haploid *S. tuberosum* x wild species hybrids (Jansky, 2006; Jansky, 2011). It is believed that nuclear-cytoplasmic male sterility can be attributed to the interactions between a dominant gene (MS) from the wild parent and factors in *S. tuberosum* cytoplasm (Camadro et al., 2004). Nuclear genes that restore fertility to interspecific hybrids have been reported (Spooner et al., 2014).

Endosperm development is critical for viable seed production in potatoes. The endosperm balance number (EBN) hypothesis assumes that a 2:1 maternal to paternal ratio of the genes controlling EBN, rather than genomes, is necessary for normal endosperm development in potatoes (Spooner et al., 2014). Consequently, successful interspecific hybridization occurs only when parents produce gametes with the same EBN, if other hybridization barriers are not present (Jansky, 2006). In potato the EBN is controlled by a few genes with a variety of alleles (Ortiz, 1998). The EBN is an arbitrary value, experimentally assigned to each *Solanum* species based on their ability to hybridize with each other (Jansky, 2006). Cultivated *S. tuberosum* has 4 EBN. Diploid species have either 1 EBN or 2 EBN. Tetraploid species have 2 or 4 EBN. All of the pentaploids and hexaploid have EBN of 4. Thus, gene flow may occur between species of different ploidy but similar EBN, whereas species of the same ploidy could be isolated from each other if they hold different EBN (Ortiz, 1998).

Crosses between species with different EBN could be manipulated when one of the species produces 2n gametes, which are gametophytes with unreduced chromosome numbers formed by inherited cytological alterations during meiosis (Larrosa et al., 2012). Meiotic mutations, which cause numerically unreduced (2n) gametes, occur naturally and frequently in cultivated and wild potatoes (Spooner et al., 2014). A cross between a tetraploid and a 2n

gamete-producing diploid will produce only tetraploid offspring. The produced 2n gametes, as a consequence of meiotic mutation could transmit a large proportion of heterozygous loci and epistatic genes to the tetraploid offspring (Peloquin et al., 2008). This allelic diversity is likely to buffer against environmental variability leading to yield diversity (Ortiz, 1998). Hence, EBN and 2n gametes, have complementary role to facilitate ploidy manipulation and thereby interspecific gene introgression.

1.6 Inheritance of genes in the cultivated tetraploid potatoes

The principal cultivated species of potato, *S. tuberosum* is an autotetraploid which displays tetrasomic inheritance (Bradshaw and Mackay, 1994). It is a highly heterozygous outcrossing species but asexually propagated, via tubers, for production and germplasm maintenance. Genetic load, which is the proportion of deleterious recessive alleles in a plant, is high in tetraploids where homozygous recessive genotypes are less common than in diploids. Hence, the crop suffer inbreeding depression when self-pollinated or crossed to genetically related clones (Spooner et al., 2014). Inbreeding depression in potatoes has impeded the elimination of unfavourable alleles and the fixation of alleles responsible for important traits (Spooner et al., 2014).

Sexual propagation and the production of 'true' seed allow breeders to generate genetic variation, and as a clonal crop, there are opportunities to exploit both additive and non-additive genetic variations in the potatoes (Paget, 2014). Theoretically an autotetraploid can carry four alleles per locus. Hence, the number of combinations within a gene and epistatic interaction among genes is much higher than can be achieved in diploids (Spooner et al., 2014). In addition, larger samples of segregating populations need to be evaluated in order to characterize genetic ratios and to identify clones carrying genes for traits of interest (Spooner et al., 2014). However, recent genomic studies have revealed that tri-allelic and tetra-allelic loci are rare in potato cultivars (Hirsch et al., 2013; Spooner et al., 2014). This calls for re-evaluation of the general requirement of intra-locus interactions, as a major concept for potato breeding (Spooner et al., 2014).

1.7 Importance of potato in Ethiopia

Potato has become an important staple and cash crop in the highlands of Ethiopia. Production and consumption of potato are increasing over time. Over the last 20 years the potato production has grown considerably, from 349,000.00 in 1993 to 775,503.00 tons in 2013 (FAOSTAT, 2015). Ethiopian agriculture is rainfall dependent subsistence farming with small land holdings (0.5 - 2 hectare) (Gebre-Selassie and Bekele, 2012). Potato yields more food per unit of land than any other major crop (FAO, 2009). In subsistence production (Haverkort et al., 2012; Kolech et al., 2015), potato has an important role as a food security

crop and to improve the livelihoods of small scale farmers who hold 95% of agricultural land in a rural community that accounts for 83% of the total population.

Potato has excellent nutritional value. In addition to its contribution to carbohydrate, calorie and quality protein in diet, it is a good source of important nutrients such as dietary fibre, vitamin B6, vitamins B3 vitamin C, iron, potassium, copper, manganese, phosphorus, carotenoids and polyphenols (Paget, 2014). Potatoes in Ethiopia are mainly consumed boiled or prepared in a stew. The consumption of potato in the forms of chips and French fries has been limited but is steadily increasing over time due to urbanization, rising middle class, and tourism (Tesfaye, 2010; Haverkort et al., 2012). Devaux et al. (2014) stated that potato cropping systems help improve resilience especially among smallholder farmers by providing direct access to nutritious food, increasing household incomes, and reducing their vulnerability to food price volatility. This makes potato an important best fit crop in reducing hunger and malnutrition of the extremely poor.

1.8 Constraints of potato production in Ethiopia

Potato productivity is relatively low in Ethiopia. The national average yield is 11 t ha^{-1} which is more than four-fold lower as compared to yield obtained in research plots (Woldegiorgis, 2013). Drought, late blight and bacterial wilt diseases are the predominant constraints limiting potato yields in Ethiopia (Kassa and Beyene, 2001; Bekele et al., 2011; Kolech et al., 2015). Under Ethiopian erratic rainfall condition, potato suffers water deficit when the season is dry and late blight disease when the season is wet in most of rain fed growing conditions (Forbes et al., 2003; Kolech et al., 2015).

1.8.1 Late blight disease

Late blight is a polycyclic disease, caused by *Phytophthora infestans* (Mont) De Bary (Turner, 2008). Potato late blight continues to be one of the most devastating plant diseases throughout the world. The disease is known for its role in causing the Irish potato famine in the 1840s. Today, the disease is still responsible for significant losses of production despite the efforts of potato breeders and fungicide producers (Grünwald and Flier, 2005; Cooke et al., 2011). Late blight was first reported in east Africa in 1941 (Nattrass, 1944) and has since continued to be devastating in the major potato growing tropical highlands of this region including Ethiopia (Olanya et al., 2006; Cooke et al., 2011).

1.8.1.1 Causal organism of late blight

P. infestans is a coenocytic (multinucleate) oomycete with diploid nuclei, which are unrelated to true fungi (Van West et al., 1999). Oomycetes lack chitin in their cell walls, and produce short lived motile biflagellate zoospores (Fry et al., 1993). The asexual reproduction of *P.*

infestans is with ellipsoid to lemon shaped spores called sporangia. Sporangia are produced on the branch tips of the alternately branched sporangiophores that grow from infected tissue (Chycoski, 1995; Fry, 2008; Kaila, 2015). Infections of foliage or tubers are initiated by sporangia either directly with a germ tube or indirectly by liberating zoospores (Harrison, 1992; Fry et al., 1993). After penetration, the pathogen forms a specialized hyphal structure, referred to as an infection vessel. Hyphae extend from this and begin colonization of plant tissue intercellularly. Intercellular hyphae form haustoria that penetrate cells to absorb nutrients. After a certain amount of time, sporangiophores grow out of stomatal openings (Guest and Brown, 1997). Where *P. infestans* exists as an asexual organism, it is essentially an obligate parasite. It requires a living host (crop debris or solanaceous weeds) for long-term survival. Sporangia may survive days or weeks in soil, whereas mycelium of the fungus cannot survive in the absence of a living host cell (Chycoski, 1995).

The pathogen is heterothallic and requires two mating types namely A1 and A2 for sexual reproduction (Wiik, 2014). Spores produced by sexual mating are called oospores (Fry, 2008). Both compatibility types must infect the same plant or tuber for oospores to be produced. Oospores have thickened walls and are believed to survive in soils for several years in the absence of living hosts (Erwin and Ribeiro, 1996; Fry, 2008). Both mating types (A1 and A2) were common in Mexico, but were apparently not common in other locations. Outside Mexico, populations of *P. infestans* were dominated by a particular clonal lineage (US-1) (Goodwin et al., 1994). But starting from the 1980s A2 mating types, isolates of A1 mating type that were quite different from US-1, began to appear in other locations worldwide. The recent distribution of the A2 mating type has had significant impacts on disease severity and incidence. Sexual reproduction has led to a genetically more diverse population of *P. infestans* that increased adaptability to host and environment (Cooke et al., 2011; Wiik, 2014). Recent studies on mating type and virulence of *P. infestans* are lacking in Ethiopia. Schiessendoppler and Molnar (2002) reported that only A1 clonal lineage mating type are present with host-specificity. Race analysis of *P. infestans* performed at Holetta Research Centre in Ethiopia showed that Race (R) 1, 2, 3, 4, and 6 were identified. Among these, R2 and R3 were the most prevalent (Kassa and Hiskias, 1994). The pathogen generally considered to have a limited host range and is a near-obligate hemibiotrophic. Potato and tomato (*Lycopersicon esculentum)* are the most economically important primary hosts of *P. infestans* (Erwin and Ribeiro, 1996; Fry, 2008; Kaila, 2015).

1.8.1.2 Epidemiology and disease cycle

Epidemic development of potato late blight critically depends on the inoculum sources, the local climate and the genetics of the host and pathogen (Grünwald and Flier, 2005; Kaila, 2015). *P. infestans* survives on tubers in storage, cull piles or other host plants. Tubers

become infected through lenticles and wounds when spores are washed into the soil by rain from infected leaves. Seed tubers play an important role in the long-distance dispersal of *P. infestans* (Fry et al., 1993). In areas where potatoes are produced around the year, the pathogen is always present; hence the disease is also always present, even though limited to only humid microenvironments. For this reason, late blight generally occurs in the tropics at the onset of the rainy season (Forbes et al., 2003). In Ethiopia, farmers practice early plantings of potatoes during the main season and off season productions of potatoes and tomatoes to avoid late blight pressure. Since the rain pattern is variable, areas wet enough for blight incidence at any time of the year could easily be found (Forbes et al., 2003; Hirpa et al., 2010).

For the disease development, temperature and humidity are of fundamental importance. The optimum temperatures for late blight disease development are near 20°C. However, the disease can occur from about 5°C to 30°C (Harrison, 1992). Sporangia germination is temperature dependent. Air temperature of 15°C appears to be a point of differentiation, below which germination is indirect through zoospore, and above which it is direct through germination tubes (Fry, 2008). Germination and zoospore activity can occur at very low temperatures, near 0°C, although at a very slow rate. Above 30°C, sporangia do not germinate and most phases of *P. infestans* cannot survive (Harrison, 1992; Mizubuti and Forbes, 2002).

Under optimal conditions (18-22°C), and with a susceptible potato cultivar, infections can be visible in three days (Mizubuti et al., 2000; Fry, 2008). Within a day or two after the lesion first becomes visible, the pathogen is capable of sporulation. Moderate temperatures (10-25°C) and wet conditions (100% relative humidity) are required for sporulation. Within 8-12 h of favourable conditions, sporangia are produced on indeterminate sporangiophores. Sporangia dislodge during changing relative humidity and can be captured in air currents or splash dispersed. As sporangia can survive for hours in unsaturated atmospheres when protected from solar radiation (Mizubuti et al., 2000), aerial dispersal up to hundreds of kilometres are possible. Under favourable conditions, massive numbers of sporangia can be produced from a single lesion (up to 300,000 sporangia per lesion) and readily dispersed. This explains why rapid development of the disease over a large area followed by complete destruction, is possible in susceptible cultivars within a few days (Harrison, 1992; Mizubuti and Forbes, 2002; Fry, 2008).

Generally, saturated air or leaf wetness is required for sporangia to germinate and for zoospore motility (Harrison, 1992). The vegetative zoospores, by which the disease commonly spread from plant to plant, are fragile, water-dependent and short-lived. After

infection has occurred, the mycelium is relatively protected from low humidity, but high ambient humidity, near saturation, is needed for sporangia formation (Harrison, 1992).

Most potato are grown in Ethiopia in a typical tropical highland climate (>1500 meter above sea level (masl)). The annual rainfall range between 600 - 1,200 mm, most of which occurs during July to September, and mean monthly temperatures ranges from 10 to 20°C with a considerable diurnal range depending on altitude (CIP, 2012). Given the precipitation and relatively cool temperatures, this climate is favourable to late blight disease development in the country (Grünwald and Flier, 2005).

1.8.1.3 Late blight symptom

P. infestans, infects potato foliage, stems and tubers (Fry, 2008). The disease appears first as water-soaked irregular pale green lesions mostly near tips and margins of leaves (Kaila, 2015). These lesions rapidly grow into large brown to purplish black necrotic spots under condition of high humidity and cool temperature. During morning hours, a white mildew consisting of sporangia and spores of the pathogen may be visible on the lower surface of infected leaves, especially around the edges of the necrotic lesions and soon the entire leaves are infected and die. In dry weather, the existing lesions turn black, curl, wither, and no mycelia appear on the underside of the leaves (Agrios, 2004).

When late blight attacks the stem it can cause girdling and the leaves wilt above the point of infection. Light to dark brown lesions on stems or petioles elongate and encircle the stems. Stem lesions become brittle and the stem frequently breaks at that point. Rain-borne sporangia from the diseased foliage can also infect tubers in the soil (Andrivon, 1995). Infection in tubers generally occurs at eyes, lenticels, or through wounds. The infected tubers show irregular reddish brown to purplish slightly depressed areas that extend deep into internal tissue of the tubers (Kaila, 2015). The infected tubers are initially hard, dry, and firm but may be invaded by other pathogens, mainly bacteria, leading to soft rot. A pungent putrid smell is often associated with heavily infected fields. This is due to rotting of dead tissue and is not a direct consequence of late blight infection (Forbes et al., 2014).

1.8.1.4 Potato late blight control measures

Reducing the primary sources of inoculum

Infected tubers, cull piles next to fields, or volunteer plants are potential sources from where inoculum could spread between potato fields. Reduction of these sources of initial inoculum can minimize the initial amount of disease or delay disease initiation. Thus, management strategies based on sanitation (removal of cull piles, elimination of volunteer plants, etc.) and the use of healthy seed tubers can improve disease control (Forbes et al., 2003). Tuber infection can be reduced through good agronomic practices such as drainage, ridging, dehulming and maintenance fungicidal protection until the haulm is completely dead. Acid soils and high aluminium availability in soils, commonly found in the tropics, have been shown to inhibit *P. infestans* in soil (Andrivon, 1995). Even though little research has been done on quantification of infected tubers in Ethiopia, there appears to be little evidence that tuber infection are major sources of inoculum in tropical highlands. As described above, many fields are planted when blight is already present and the relative role of aerial inoculum from sporangia coming from foliage infection would seem much more significant. Given the limited clean seed source, presence of dumps, volunteer plants and year round cultivation of solanaceous hosts, control of aerial and tuber inoculum is not easy. This leaves the control measure to depend much on fungicides and genetic resistance measures (Forbes et al., 2003).

Fungicides

Fungicide use is the most common practice for late blight control worldwide. However, its use increases production costs and has negative consequences for environment and human health. A survey made by Kolech et al. (2015) showed that more than 88% of farmers in central parts of Ethiopia use fungicides, while farmers in four major potato producing districts in Amhara region, do not spray at all to control late blight. This region contribute over 40% of the total production in the country. The limited fungicide use is mainly because of the cost involved.

For an efficient and cost-effective use of fungicides, adequate knowledge on the type, dosage, frequency and timing of application are required. Fungicide and variety reaction studies conducted in Uganda, Kenya and Ethiopia suggests that significant late blight control can be achieved when the protectant fungicide, mancozeb is applied on a scheduled basis (Olanya et al., 2001). On-farm research also indicates that three timely applications of a protectant or a protectant fungicide alternated with a systemic fungicide can be effective for late blight management (Olanya et al., 2001). However the number of fungicide applications needed is strongly affected by the weather conditions of each year. To start spraying

fungicides at the right time, a disease forecasting system is important. However, this system requires weather-measuring devices and/or computers, which are not available for resource-poor farmers (Forbes et al., 2003).

The most widely available fungicides in the market in Ethiopia are those products containing mancozeb, metalaxyl, and chlorothalonil compounds as active ingredients (Haverkort et al., 2012). Fungicides with metalaxyl active ingredient have a strong curative effect. The main disadvantage of these fungicides is that resistance to the fungicide readily develops in the pathogen population. There is limited information concerning the status of metalaxyl resistance, but CIP (2004) stated that 20% of the collected isolates were resistant. The resistance expected increases as the rate of application is increased since *P. infestans* is capable of quick development of resistant strains. Control of primary inoculum sources, use of dynamic fungicide dosages related to weather forecasts and exploitation of resistant cultivars, would reduce financial costs and unfavourable environmental consequences (Forbes et al., 2003). Although economical control of late blight disease has been achieved with timely use of effective fungicides, the use of fungicides adds a huge cost to potato production which is not affordable especially for Ethiopian small scale farmers. In addition, wide use of fungicides may also create health problems for users, adversely affect the environment, and result in the selection of fungicide resistant strains of the pathogen. Therefore, breeding for cultivars with durable and adequate-level resistance for potato crop is the most effective, economical, and environmentally friendly method of disease control.

1.8.1.5 Breeding potato for late blight resistance

Past breeding efforts for late blight resistance in Ethiopia

In Ethiopia, limited knowledge and financial inputs to apply fungicides, and limited supply of clean seed makes genetic control of late blight an important objective of the potato breeding programme (Colon et al., 1995b; Woldegiorgis, 2013). With the objective of developing late-blight resistant cultivars and other economically important traits, research has been undertaken in Ethiopia since 1975. From 1987 to 2010, 29 improved cultivars with late blight resistance and with high yield and good quality traits were released (Woldegiorgis, 2013).

A potato breeding scheme begins with the evaluation and selecting appropriate parents, the crossing of the selected parents and the selection of elite clones from these progeny of crosses for further testing and potential release as cultivars (Paget, 2014). A narrow genetic base has been the main bottle neck for the crop improvement in the country, because of few introduction of potato cultivars (Haverkort et al., 2012). Therefore, the Ethiopian breeding program has benefited from the global collaborative efforts in potato breeding for widening the genetic base. In the past the parental selection and progeny generation has been carried

out at the International Potato Centre (CIP) in Peru, where large genetic diversity of the crop maintained. The selection in the country were performed in advanced clones, tuber families and true potato seeds received from CIP (Woldegiorgis, 2013).

Searching for durable resistance

Durability of resistance is the first concern in late blight resistance breeding (Umaerus and Umaerus, 1994). *P. infestans* is known to be a highly variable pathogen which easily adapts to host resistance based on major (R) genes which confer race specific resistance. Race specific resistance is governed by a single or few dominant genes with major effects and a clear, discontinuous segregation of progeny. Race-nonspecific or horizontal resistance, in contrast, is governed by minor genes, with small cumulative effects and segregation generation showing continuous distribution. The latter is more durable because it is polygenically controlled and shows quantitative resistance (Solano et al., 2014). Unlike race specific (qualitative) resistance, quantitative resistance permits invasion of the pathogen and its development in the host tissue in a restricted way and makes no distinction among the races of *P. infestans* (Landeo, 2002). Quantitative resistance has been described to consisting of components of resistance relative to the pathogen such as slow infection rate, slow growth and development, delayed spore's latency period and reduced spore production (Dorrance et al., 2001). This kind of resistance may be affected by environmental conditions, inoculum potential, disease progress and physiological changes of the host plant (Kaila, 2015). This could suggest the need to evaluate and select the genotypes in the target environments.

Most of the potato genotypes that have been developed and released in eastern Africa before 2008 either have genes for vertical resistance to late blight or have been developed for horizontal resistance to late blight in the presence of unknown resistance (major R) genes (Woldegiorgis, 2013). As a consequence, considerable number of the varieties have become susceptible to late blight and, hence, they are no longer produced (Mohammed, 2014).

Starting from 1990, CIP began a program to improve potato populations by increasing gene frequencies for quantitative (horizontal) resistance to late blight together with systematically upgrading and maintaining other farmer and consumer preferred traits. This was done by removing known dominant genes responsible for race-specific resistance, which could mask the quantitative resistance and using the recurrent selection scheme by crossing the best genotypes of the population with each other (Landeo et al., 2001). Population B3 has mostly *S. demmisum* derived horizontal resistance genes improved mainly from *S. tuberosum* ssp. *tuberosum* germplasm background (Landeo et al., 2001) and is adapted to tropical highlands. This population is currently available as a source of breeding material. Population

B3 has shown stability of resistance to diverse environments and pathogen populations in tropical environments (Landeo, 2002).

Screening and evaluation for late blight resistance

Screening of germplasm for resistance to late blight may be done both in the laboratory and in the field. In the latter condition, screening can be done in different locations with high disease pressure by planting the materials to be tested along with known susceptible and resistant check cultivars. Screening potato germplasm for late blight resistance can also be achieved in controlled environments using laboratory methods utilizing leaf disks, detached leaflets or detached leaves (Sleper and Poehlman, 2006).

Field screening is generally done on a large population of plants. For this purpose disease should be recorded in each cultivar right from its appearance till the maximum build-up of the disease at regular (weekly) intervals. This data would allow to calculate the area under disease progress curve (AUDPC) (Wilcoxson et al., 1975; Shaner and Finney, 1977), which has been considered more reliable for categorizing the cultivars according to their resistance grades. AUDPC integrate all aspects of disease progress in relation to host development and growth. AUDPC has been widely used for field assessment of quantitative resistance (Jeger and Viljanen-Rollinson, 2001; Wulff et al., 2007; Kaila, 2015).

1.8.2 Drought in Ethiopia and breeding for tuber yield under moisture-stressed environment

Ethiopia is highly vulnerable to the impacts of frequent droughts, exacerbating the existing challenges to satisfy the food demands due to the increasing population (Tadesse et al., 2014). Studies show that drought frequency and intensity is increasing over past few decades in a large geographical area of Ethiopia including the most populous and arable highlands (Deressa et al., 2014; Teklu, 2014). About 55% of the total land area constitutes moisture-stressed areas with crop growing period of less than four months (Teklu, 2014). Drought in Ethiopia can reduce household farm production by up to 90% of a normal year's output and lead to the death and migration of humans (Deressa et al., 2014). About 85% of the population is rural, who depend mainly on rain-fed agriculture. Thus, vulnerability to poverty persists in rural households because of their poor coping capacity, which draws attention to the need for new and improved agricultural technologies (Teklu, 2014). Genetic enhancement will increase productivity of crops and food availability. Potato can make important contribution in this aspect.

1.8.2.1 Potato as efficient water user but drought sensitive crop

Under rain-fed conditions, potato yields more food per unit of water than any other major crops. For every cubic meter of water applied to the crop, potato produces 5600 kcal of dietary energy, compared to 3860 in maize, 2300 in wheat and 2000 in rice (FAO, 2009; Monneveux et al., 2013). Because of its high productivity, potato is regarded as a food security crop. The crop is, however, considered a drought sensitive as compared to most other crop species (Deblonde and Ledent, 2001). Vayda (1994) has also confirmed that potato yield is especially sensitive to drought. Drought can affect potato growth and production by reducing the amount of productive foliage, by decreasing the rate of photosynthesis per unit of leaf area and by shortening the vegetative period (Van Loon, 1986; Spitters and Schapendonk, 1990). Potato exhibits morphological changes at -0.4 bar, that is, when soil moisture only drops to 70-85% of field capacity, depending on the relative humidity (Vayda, 1994). Leaf expansion of the potato plant declines at a mild water stress level, a leaf water potential of -3 bars (Van Loon, 1986; Jefferies, 1995). Plants under water stress will close their stomata leading to a decrease in photosynthetic rate, which results in yield reduction. Stomatal closure enhances the differences between canopy and air temperature (Dalla Costa et al., 1997).

Drought in the period of tuber initiation and bulking has the most drastic effect on yield (Vayda, 1994). Water stress during the tuber bulking period encourages plant senescence, resulting in a decrease in leaf area index (LAI). At first the lower leaves start to wilt and abort. Simultaneously drought inhibits the development of new leaves (Van Loon, 1986). However there are varietal differences in yield and yield component responses to moisture stress (Lynch et al., 1995).

1.8.2.2 Yield components and yield determinant

The primary objective in breeding potato is increased tuber yield. No new variety has any chance of succeeding unless it is at least as high yielding as the present varieties (Howard, 1992). Total yield of a potato crop depends up on number of plants per unit area, number of tubers produced per plant and mass of the individual tubers. These variables are dependent on one another (Sleper and Poehlman, 2006). The number of tubers is highly correlated with the number of stems produced while a negative relationship exists between number of stems per plant and number of tubers per stem. The amount of foliage present has a strong influence on tuber yield. A leaf area index (LAI) of 3 occurs when 95-98% of incident radiation is intercepted, after which the correlation between tuber yield and leaf area decreases (Gregory and Simmonds, 1992; Howard, 1992).

1.8.2.3 Screening for drought tolerance

Traits conferring yield stability under drought might include an array of morphological, physiological and biochemical adaptations involving hundreds of genes. Drought tolerance traits increase plant vigour and survival rate under water-limiting conditions (Schafleitner et al., 2007). In potato, maintenance of a high photosynthetic rate under drought has been proposed as the most crucial drought tolerance trait. Less reduction of stomatal conductance and rooting depth have also been associated with drought tolerance (Schafleitner et al., 2007). However, observation of the root system in early generation is tedious and requires labour and time. Some traits, such as small plant size, reduced leaf area, early maturity and prolonged stomatal closure lead to a reduced total seasonal evapotranspiration resulting in a reduced yield potential (Van Loon, 1981; Cattivelli et al., 2008).

Yield performance has been used as the most important criteria for screening cultivars under moisture stress condition (Alsharari et al., 2007). Most studies estimated drought susceptibility index (DSI) by comparing yield performance of a given genotype under moisture stress and well-watered conditions. This is to differentiate between genotypes that have high yield under drought stress simply because of high inherent yield potential and those that also have greater drought tolerance *per se* (Yadav and Bhatnagar, 2001; Cabello et al., 2012).

Canopy temperature measurements have been widely used to study the drought response of various crops. This approach is based upon the close, inverse relationship between leaf temperature and transpirational cooling. High canopy temperature related to high stomatal resistance (Blum, 1988) and thereby reduced photosynthetic rate, leading to a lower yield level (Blonquist Jr. et al., 2009). As stomates close in response to soil water depletion and a decrease in water uptake, plant temperature increases. Consequently, photosynthesis is reduced because CO_2 absorption is reduced (Blonquist Jr. et al., 2009). Blum et al. (1989) found a positive correlation between drought susceptibility of wheat genotypes and canopy temperature in stressed environments. Genotypes that suffered greater relative yield losses under drought stress tended to have warmer canopies at midday. Stark et al. (1991) also reported that the most drought resistant potato genotypes usually had the lowest canopy temperatures during periods of drought.

Kumar and Singh (1998) found in oil seed *Brassica* species that lower canopy temperature positively correlated with osmotic adjustment. Plants with higher osmotic adjustment transpired more water and therefore had cooler canopies than the plants with lower osmotic adjustment. Osmotic adjustment is an important mechanism enabling plants under water stress to maintain water absorption and cell turgor pressure, thus contributing to sustained

higher photosynthetic rate and expansion growth (Cattivelli et al., 2008). Osmotic adjustment involves the net accumulation of solutes in a cell in response to a reduced water potential of the cell's environment. Because of the accumulation of solutes, the osmotic potential of the cell is lowered, which in turn attracts water into the cell and tends to maintain turgor pressure (Blum et al., 1996).

The amount of intercepted radiation is a major factor which influences the final tuber yield of potato crops under drought conditions (Deblonde and Ledent, 2000). Measuring the proportion of ground covered by green foliage (ground cover) is a measurements of crop canopy development and used to estimate light interception and carbon acquisition (de la Casa et al., 2007). Deblonde and Ledent (2000) reported genotypes with high ground cover tended to have high intercepted radiation and high tuber yield. Boyd et al. (2002) and de la Casa et al. (2007) showed high correlation between ground cover and leaf area index. Thus, it can be used as an important parameter for discriminating cultivars according to their yielding ability under moisture stress.

Stay green has become a noted trait in breeding programs, especially in environments where terminal drought is the main recurrent problem, as it indicates reduced chlorophyll degradation or delayed senescence (Cattivelli et al., 2008; Blum, 2011; Rolando et al., 2015). Leaf senescence is a highly organized and well-regulated process, in which chlorophyll and foliar proteins tend to degrade at similar rates (Rolando et al., 2015). Plant hormones are tightly linked to senescence control, where abscisic acid (ABA) promote senescence and kinetin delays it. Ethylene, is a known accelerator of senescence mediated by ABA (Blum, 2011). Jensen et al. (2010) hypothesized that the delayed senescence could be caused by a possible negative effect of ABA concentration in the ethylene production. Under water restriction, delayed senescence (or stay-green) may reflect a maintenance of photosynthetic activity, which seems to be preferred by breeders (Blum, 2011; Rolando et al., 2015). However, Blum (2011) pointed out that stay green trait might delay remobilization of carbon products (stem reserves) to the harvested organs of the plant, which might lead to a lower yield. In potato Rolando et al. (2015) observed increased leaf greenness under water restriction, which seems to be associated with a decrease in leaf growth or turgor loss. The highest increment in leaf greenness in the above study observed on drought susceptible genotypes. Ramírez et al. (2014) also reported that stay green were negatively correlated with tuber yield under water stress in potato cultivars.

1.9 Cultivar development strategies in potato

1.9.1 Broadening the genetic bases

Identification of superior parents with high yield and desirable traits is the basis of the breeding program. Wider genetic base is important to choose the best parents for breeding, to design proper crossing schemes and selection strategies (Carputo et al., 2013). As modern potato breeding was started with only a few genotypes, narrow genetic base and limited accessibility to available genetic variation remains a challenge of potato breeding (Paget, 2014; Mihovilovich et al., 2015). Wild potatoes have contributed many genes of interest to potato breeding (Gebhardt and Valkonen, 2001). Among these, for example *S. demissum* is a widely used hexaploid, which has been used as a source of the major R gene as well as minor genes (for Population B3) that confers resistance to late blight (Acquaah, 2007). However, only a small number of wild species have been used extensively, compared with the huge natural genetic diversity available in the wild relatives of the potato (Bradshaw et al., 2006). As potato is a heterozygous out breeder, use of the same recurrent parent during introgression would result in a self of the recurrent parent and hence inbreeding depression (Colon et al., 1995a; Khiutti et al., 2015). Genetic breeding barriers (see section 1.5) associated with many wild potato species are the major challenges to exploit wild germplasm (Weber et al., 2012). Ploidy manipulations and bridge crosses, mentor pollination, embryo rescue and hormone treatment have been the methods used to overcome the difficulty of hybridization between sexually incompatible species (Jansky, 2006). Understanding these barriers and developing techniques to allow for introgression are fundamental to utilize the wealth of genetic resources in potato (Jansky, 2006; Weber et al., 2012).

Potato germplasm resources provide genes for biotic and abiotic stress resistance, processing quality, and nutritional value (Bradshaw et al., 2006; Jansky, 2006). Resistance for late blight has been identified within and among several species of wild *Solanum*, that may or may not be sexually compatible with cultivated potatoes (Khiutti et al., 2015). Cabello et al. (2012) reported more drought tolerant genotypes in polyploid species and cultivar groups than the diploid ones. The authors pointed out Andean potatoes (*S. tuberosum*) potatoes, as a potential source of drought tolerance. Wild *Solanum* species add allelic diversity that can contribute to hybrid vigour and phenotypic stability (Darmo and Peloquin 1990). Careful selection of both cultivated and wild species parents can result in a large proportion of fertile and economically desirable hybrid offspring after several cycles of recurrent selection (Jansky, 2011; Paget, 2014).

1.9.2 Selection for potential parents

Since the cultivated potato is tetraploid (2n = 4x = 48) and highly heterozygous, crosses are made between parents with complementary features as selection of parents is based on phenotype rather than genotype (Bradshaw and Mackay, 1994). In recent years, major progress has been made in the use of molecular technologies for the identification of genes to enable the implementation of marker-assisted selection in crop improvement programs (Slater et al., 2014). This may help to accelerate future potato breeding. Conventional breeding, which is the main method to develop new improved cultivars include hybridization and selection (Bradshaw, 1994; Sleper and Poehlman, 2006). The procedure starts with identification of desirable parents, followed by crossing selected superior genotypes for the trait under consideration. The approach used by potato breeders for selection of desirable parents include use of mid-parent values, combining ability effects, estimated breeding values, progeny tests and genetic diversity analysis (Gopal, 2015).

1.9.2.1 Selection based on combining ability

Combining ability is a type of progeny test which predicts the performance of parents in crosses. This is the factor determining genotype's potential for cultivar development (Hallauer et al., 1988; Acquaah, 2007). Combining ability effects are partitioned in to two types: general combining ability (GCA) and specific combining ability (SCA). The GCA depends predominantly on additive effects of the genes. Where GCA predominates, progeny performance can be reliably predicted from the performance of the parents. The SCA effect depends predominantly on non-additive effects of the genes. Then, when the SCA effect predominates specific combinations of parents would have to be evaluated to find progeny with the desired characteristic and considerably more testing would be required (Machado et al., 2002). Diallel and North Carolina Design II are the most widely used mating designs for an efficient estimation of combining ability effects (Acquaah, 2007).

Variable reports on the importance of GCA and SCA effects for late blight resistance exist in the literature. These conflicting views may be the result of using different mating designs and/or different genetic parent materials for evaluation (Haynes et al., 2008). Despite the differences, studies showed that both GCA and SCA effects are important for choosing blight resistant parents for use in breeding (Tai and Hodgson, 1975; Haynes et al., 2008). Potato breeding work in Ethiopia focused on introduction and testing of advanced clones developed by CIP for late blight resistance and productivity. However, little work has been done in identifying parents, and generating and selecting progenies which combine drought tolerance and resistance to late blight (CIP, 2012).

1.9.2.2 Genetic diversity assessments

Genetic diversity study is another important method to choose the best parents for breeding (Carputo et al., 2013). High level of genetic diversity serves as insurance against crop failure due to biotic and abiotic stresses (Fregene et al., 2003). In potato breeding, it has been assumed that hybrid vigour is maximized by using genetically divergent parents for crosses. However, maximizing heterozygosity should be coupled with the presence of certain alleles responsible for the desired traits, in order to realize genetic gain in a population derived from genetically divergent parents (Bonierbale et al., 1993). There are several molecular tools that are used for the genetic diversity study. Simple sequence repeat (SSR) markers are among the most widely used, owing to their high specificity, high polymorphism, good reproducibility and high throughput (Tenzer et al., 1999; Carputo et al., 2013; de Galarreta et al., 2013).

1.9.3 Selection of elite clones

In potato, genetic variation is achieved in the F1 generation following hybridization (Bradshaw, 1994; Acquaah, 2007). Potato heterozygosity is fixed by asexual reproduction using tubers. As such, F1 progenies obtained from crosses between two genotypes are each genetically unique and show a range of phenotypes (Mori et al., 2015). Highly heritable traits will be selected at early breeding stage, while selection for quantitative traits that are affected by environments would be performed at latter stage (Mori et al., 2015). Subsequently, selected superior genotypes are vegetatively propagated and maintained in their original genetic state (Bradshaw, 1994; Acquaah, 2007). Tubers harvested from each superior F1 family are grown in rows for evaluation, and then the amount of seed tubers increased for subsequent selection generations. Each row represents a clone from a single F1 plant (Bradshaw and Mackay, 1994). Selected clones are tested in multi-location trials for evaluation and subsequent selection for wide or specific adaptation and yield stability before release (Sleper and Poehlman, 2006).

As a clonally propagated crop, potato has the disadvantage of low multiplication rate, which can increase evaluation period and disease vulnerability, which can be transmitted by infected seed tubers such as viruses, insects, fungal and bacterial pathogens (Gebhardt and Valkonen, 2001). Infected seeds can transmit the disease for the next season and causing progressive yield and vigour degeneration, further restricting the germplasm use in breeding efforts (Hirsch et al., 2013). Thus, special attention should be given for germplasm ‘maintenance’ breeding in potato breeding program, which require intensive resources.

1.10 Conclusions

Potato has become increasingly important crop in eastern African highlands due to its adaptability, yield potential, and nutritional advantages relative to other crops. However, new potato cultivars with increased yield and improved performance under biotic and abiotic stress are needed to keep up with increasing food demands and the effects of climate change. This chapter reviewed important aspects of breeding potato particularly for late blight resistance and drought tolerance. The review highlighted the availability of resistant materials for both traits from cultivated and wild relatives of potatoes and their use is dependent on manipulation of crossing barriers. Selection of parents based on their combining ability and their genetic distance is an efficient method to achieve genetic gains. Traits used to screen drought tolerance and late blight resistance are summarised. There is limited recent information published in Ethiopia on the variability of *P. infestans* and studies related to drought in potatoes, which call for research in these areas.

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CHAPTER 2. Response of potato clones to late blight disease, yield and yield related traits in north-western highlands of Ethiopia

Abstract

Late blight disease of potato caused by *Phytophthora infestans* poses a significant threat to potato production in Ethiopia. High yielding genotypes with adequate late blight resistance remain a strong component in integrated disease management strategy. Several potato cultivars released in Ethiopia have succumbed to late blight disease requiring new sources of resistance for breeding. The objective of this study was to select late blight resistant and high yielding potato clones under field condition in north-western Ethiopia. Twenty four clones (17 B3C2 population acquired from the International Potato Centre and 7 widely grown released and farmers' cultivars), were evaluated at three locations. The experiments were planted in north-western Ethiopia using a randomized complete block design with two replications. Data collected included area under disease progress curve (AUDPC), days to 5% severity threshold, relative yield loss percentage, total tuber yield, marketable tuber yield, total tuber number and marketable tuber number. Results showed that clones differ significantly for all traits assessed across locations. The following five clones combine high to moderate resistance to late blight with high yields: 396029.25, 395017.229, 396004.263, 396034.103 and 395077.12. These clones, all from B3C2 population, are useful genetic resources for resistance breeding against late blight disease and for enhanced yields.

Key words: highland tropics, tetraploid potatoes, resistance breeding, yield loss, AUDPC.

2.1 Introduction

Ethiopia is among the leading ten sub-Saharan Africa countries in terms of areas of potato production (FAOSTAT, 2015). Potatoes are a source of both food and cash income in the densely populated highlands of the country which is inhabited by 90% of the population (Gildemacher et al., 2009; Chindi et al., 2013). This makes potato a high-potential contributor to national food security (FAO, 2009; Gildemacher et al., 2009). However, the national average yield of the crop is less than 11 t ha^{-1} which is far below the attainable yield of 45 t ha^{-1} (Berihun and Woldegiorgis, 2013; Chindi et al., 2013; FAOSTAT, 2015). Of the constraints that widen the gap between actual and attainable yield, late blight is the most serious disease (Fuglie, 2007; Gildemacher et al., 2009; Forbes, 2012; Sparks et al., 2014).

Potato late blight disease, caused by the heterothallic oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, is a major threat that can cause complete crop failure (Trognitz et

al., 2001; Fry, 2008). Yield losses of 30 to 100% were reported in Ethiopia (Kassa and Beyene, 2001; Berihun and Woldegiorgis, 2013). The disease damages leaves, stems and tubers and is most devastating throughout the major potato producing areas in the country (CIP, 2004; Villamon et al., 2005; Forbes, 2012; Woldegiorgis, 2013). Research showed that the population of *P. infestans* in Ethiopia has the A1 mating type (US 1 clonal lineage), which reproduces asexually (Schiessendoppler and Molnar, 2002).

Effective control of late blight disease requires integrated disease management (Mundt et al., 2002). The disease can be controlled by the application of fungicides, cultural practices such as early planting, eliminating the source of inoculum, and/or using resistant cultivars (Garrett et al., 2001). However, deployment of these methods individually could not provide sufficient control of the disease. Fungicides can provide good control but they are often unaffordable for the small scale farmers, who account for over 90% of potato crop production in Ethiopia (Mizubuti and Forbes, 2002; Schulte-Geldermann, 2013). Also, fungicides are unsafe to human health and the environment. In some parts of Ethiopia, farmers plant potatoes early in the dry season to escape heavy late-blight pressure, though yield levels are compromised due to insufficient soil moisture (Forbes et al., 2003). Additional factors that contribute to high levels of late blight infection are: lack of certified clean seed, monocropping practiced by most farmers, and the fact that the tubers are left in the soil for extended period (Chindi et al., 2013). Optimal management of potato late blight can best be achieved by incorporating durable resistance genes against virulent races of the fungus (Colon et al., 1995; Trognitz et al., 2001; Forbes, 2012; Woldegiorgis, 2013). This approach can be suitably integrated with other measures that fail to provide full control in isolation.

Durability of host resistance is the main concern in late blight resistance breeding (Umaerus and Umaerus, 1994). Late blight resistance can be conditioned by race specific and race non-specific or field resistance genes. It is well known that race-specific or vertical resistance is controlled by major genes. Several major genes have been identified in differential potato cultivars (Sleper and Poehlman, 2006). However the emergence of virulent pathotypes of the pathogen could rapidly overcome the resistance of one or few major genes. Consequently, the use of major genes in breeding for resistance to late blight is mostly not recommended (Haynes et al., 2008; Forbes et al., 2014). Conversely, race non-specific or field resistance, is conditioned by minor genes (Trognitz et al., 2001; Andrivon et al., 2006). Race non-specific resistance might not confer absolute protection, but is considered to be more durable than race-specific resistance, and is attributed to polygenically controlled quantitative resistance. Hence, this form of resistance is effective against a broad range of pathotypes of *P. infestans* (Bradshaw and Bonierbale, 2010).

In Ethiopia several improved potato cultivars with considerable resistance to late blight have been released to potato growers. However, a number of these cultivars have lost their resistance over time as the apparent resistance to late blight was overcome by the virulent pathotypes (Schulte-Geldermann, 2013). Advanced resistant breeding populations and candidate clones have been developed by the International Potato Centre (CIP) for a variety of agro-ecological zones including tropical highlands (CIP, 2012). This germplasm can serve as a valuable source of genetic variation in breeding programs. Among these clones, 'population B recombination cycle 3 (Pop B3)', which lacks any known major or R genes (R1 to R11) against *P. infestans*, is the latest advanced source released by the CIP for durable late blight resistance (Landeo et al., 2001; Yao et al., 2011). The population is constantly monitored to maintain sufficient genetic variation to ensure further progress and selection of outstanding clones with high levels of resistance and varietal potential (Landeo et al., 2001; Gastelo et al., 2014). Some of the clones derived from this population showed promising performance in Ethiopia and of these CIP-393371.58 has been released under the name 'Belete' in 2009 (CIP, 2012).

Potato breeding in Ethiopia has generally focused on introduction and testing of advanced clones developed by CIP for late blight resistance and productivity. However, little work has been done locally in parent selection and breeding of potato against late blight resistance (CIP, 2012; Woldegiorgis, 2013). Therefore, the objectives of this study was to select late blight resistant and high yielding potato clones under field conditions in north-western Ethiopia for breeding.

2.2 Materials and methods

2.2.1 Plant materials

The study used 24 potato genotypes. Seventeen clones were obtained from CIP, and seven were locally released and farmers cultivars widely adapted to the mid- and high-altitude environments (>1500 meter above sea level) in Ethiopia (Table 2.1). The clones sourced from CIP are from population B group three, cycle two (B3C2) which are known for their quantitative late blight resistance. Locally released clone 'Guassa', a moderately susceptible cultivar, was used as a comparative control.

Table 2.1 List of potato genotypes used in the study

No[a]	Genotype	Pedigree	Reported late-blight reaction[b]	Population
1	392633.64	387132.2 x 387334.5	Resistant	B3C2
2	393220.54	381400.22 x 387170.9	Resistant	B3C2
3	395011.2	393085.5 x 392639.8	Resistant	B3C2
4	395015.6	393083.2 x 391679.12	Moderately resistant	B3C2
5	395017.14	393085.13 x 392639.8	Moderately resistant	B3C2
6	395017.229	393085.13 x 392639.8	Resistant	B3C2
7	395077.12	391586.109 x 393053.6	Resistant	B3C2
8	395096.2	393085.5 x 393053.6	Moderately resistant	B3C2
9	395109.34	391589.26 x 393079.4	Resistant	B3C2
10	395112.32	391686.15 x 393079.4	Moderately resistant	B3C2
11	396004.26	391002.6 x 393382.64	Moderately resistant	B3C2
12	396029.25	392633.54 x 393382.64	Resistant	B3C2
13	396031.108	392633.64 x 393382.64	Resistant	B3C2
14	396034.103	393042.5 x 393280.64	Resistant	B3C2
15	396038.101	393077.54 x 393280.64	Moderately resistant	B3C2
16	396038.105	393077.54 x 393280.64	Moderately resistant	B3C2
17	396038.107	393077.54 x 393280.64	Moderately resistant	B3C2
18	Belete (393371.58)	387170.16 x 389746.2	Resistant	B3C2
19	Gorebella (382173.12)	380088.4 x MEX BULK	-	Pop A
20	Guassa (384321.9)	380479.15 x 3 BULK	Moderately susceptible	Pop A
21	Jalene (384321.19)	380479.15 x 3 BULK	-	Pop A
22	Shenkola (KP-90134.5)	382132.14 x XY.13	Moderately susceptible	Pop A
23	Gudene (386423.13)	-	Moderately resistant	Pop A
24	Aba Adamu	Farmer's cultivar	-	-

[a] Genotypes from 1 to 17 are acquired from the International Potato Centre, while 18-23 are locally released cultivars and 24 is a farmers' cultivar; [b] The information for B3C2 clones was cited from the CIP's website (http://www.cipotato.org).

2.2.2 Study sites

The study was carried out at three selected locations in north-western Ethiopia: Injibara, Adet and Debark during the main cropping season (June to October 2014). These sites represent the main potato production areas in the north-western Ethiopia. The sites are hot spot areas and experience high late blight pressure during the rainy season. Injibara (10°57′ N, 36°56′ E) is located at an altitude of 2568 meters above sea level (masl). The mean annual temperature and rainfall are 15 ºC and 1700 mm, respectively. The soils at this site are predominantly Nitosol (Shibabaw et al., 2014). Adet (11°17′ N, 37°47′ E) is situated at an altitude of 2240 masl and receives a mean total annual rainfall of 1238 mm with mean annual temperature of 17 °C, with mainly red brown Nitosol soils (Zegeye et al., 2010). Debark (13° 14′ N, 37°89′E) is situated at an altitude of 2836 masl and receives a mean total annual rainfall of 974 mm with a mean annual temperature of 12.4 °C. It has predominantly Luvic Andosols soils (Assen and Tegene, 2008). All the three locations have a monomodal rainy season which occurs between May and October except Injibara with rainy months extending from March to end of November (Shibabaw et al., 2014).

2.2.3 Seed potato preparation and experimental set up

Healthy plantlets of 17 clones acquired from CIP along with six released and one farmers' (Aba Adamu) local cultivars were multiplied in the tissue culture laboratory of Amhara Agricultural Research Institute (ARARI). These were transplanted to a screen house and harvested in June 2013. Harvested tubers were kept in diffused light storage (DLS) system for four months to break dormancy. Tubers were planted for further multiplication in November 2013 under virus free condition (cold highland and using chemical control). Tubers were then harvested and kept for four months prior to planting for the field tests.

A total of 24 entries were planted in the field during the rainy season. Two spray regimes, (sprayed and unsprayed) were used at Injibara and Adet locations for comparative study. The two treatments were arranged in separate experiments. The distance between the experiments was 3 m. The trials were established using a randomised complete block design with two replications per each spray regime and each location in June 2014. In the unsprayed treatment genotypes were exposed to natural infection using spreader rows of a susceptible local cultivar 'Enatbeguaro' to keep a continuous infection pressure during the period of disease assessment. Neither pesticides nor fungicides were applied in this regime except in Adet where late blight occurred early (two weeks after planting). In this location a contact fungicide (Mancozeb) was applied once in the second week after planting to maintain the genotypes. In the control or sprayed treatment Ridomil MZ 72 (8% a.i. metalaxyl + 64% a.i. mancozeb), Bravo (82.5% WP Chlorothalonil), Tanos (250 g kg^{-1} cymoxanil, 250 g kg^{-1} famoxadone) and Mancozeb (80% WP) were sprayed at weekly interval alternately as per the recommendation of the manufacturer. Spraying started from two weeks after planting and continued until the end of the season. At Debark, however, genotypes were evaluated only under unsprayed condition because of seed shortage.

Each genotype was represented by an experimental unit consisting of 40 plants established in a plot of 9 m^2 with 4 rows, 3 m long, with 0.75 m inter- and 0.3 m intra-row spacing. All necessary agronomic practices such as weeding and ridging were carried out by using hoe and hand cultivation. Phosphorus fertilizer in the form of diammonium phosphate was applied at the rate of 69 kg ha^{-1} and nitrogen at 81 kg ha^{-1} in the form of urea. The entire dose of phosphorus and half rate of the nitrogen fertilizers were applied at planting and the other half of nitrogen was added 45 days after planting.

Data collection

Data collected included percentage of leaf area affected by late blight, from which area under disease progress curve (AUDPC) and days to 5% disease severity threshold were

calculated, relative yield loss percentage, total tuber weight, marketable tuber weight, total tuber number per plant and marketable tuber number per plant.

Area under disease progress curve (AUDPC)

Late blight disease severity was recorded visually as percentage of foliage affected at weekly intervals starting with the first appearance of the symptoms until the susceptible control had reached 100% infection. The percentage of late blight affected leaf area per plot was estimated using a scale comprising 9 classes, corresponding to 0.01, 0.1, 1, 5, 25, 50, 75, 95 and 100% of diseased leaf tissue (Fry, 1978; Niks et al., 2011). For all plots and assessment dates, the area under the disease progress curve AUDPC (Campell and Madden, 1990) was calculated using the following formula:

$$AUDPC = \sum_{i=1}^{n-1}[(t_{i+1} - t_i)(y_{i+1} + y_i)/2]$$

Where "t" is the time of each reading, "*y*" is the percentage of affected foliage at each reading and n is the number of readings.

Area under disease progress curves were standardized to give relative area under the disease progress curve (rAUDPC) by dividing the AUDPC by the maximum potential AUDPC (Fry, 1978) to allow for comparison between different locations. The maximum potential AUDPC is calculated by multiplying the total number of days between the first and last readings by 100 as shown in the formula below.

rAUDPC = AUDPC/(Last reading day-First reading day)x100

An interval susceptibility scale (0 to 9) was calculated as described by Yuen and Forbes (2009) using the rAUDPC value resulting in low values for resistance and high values for susceptible ones. In order to use this scale, the cultivar Guassa was assigned a susceptibility value of 6 based on its moderate susceptibility.

Days to 5% disease severity threshold (DT_5)

The number of days after planting for the plants to reach the 5% disease level for each plot was also estimated and assigned as days to 5% disease severity threshold (DT_5) as proposed by Dorrance et al. (2001). The measurement of DT_5, could include the major components of partial resistance such as infection efficiency, latent period and lesion growth rate (Dorrance et al., 2001; Pariaud et al., 2009). This makes DT_5 an important parameter

especially under natural epidemics under field condition, where it is a difficult task to quantify inoculum dosage or control timing of inoculation (Dorrance et al., 2001).

Yield and yield related traits

At harvest, yield was measured for each plot. Total tuber yield was calculated by converting the total weight of all the tubers harvested in a plot in t ha^{-1}. Total tuber yield from sprayed plots was compared with those from the unsprayed plots to obtain relative yield loss. And percentage relative yield loss (RYL%) was calculated as the ratio of the difference between the yield obtained from sprayed control and the unsprayed plots to the yield of the sprayed control as shown in the formula below:

RYL%=(tuber yield of the sprayed plot-yield of unsprayed plot)/(yield of sprayed plot)×100

Tubers of each plot were graded in to three categories: >30 mm (marketable), <30mm (unmarketable), and rotten and diseased (discarded) and were counted and weighted in kg. Form the above grading, the marketable tubers yield was expressed in t ha^{-1}, number of total tubers per plant and number of marketable tubers per plant were calculated. The relative reduction of marketable tuber yield, total tuber number and marketable tubers number was also calculated as RR = (sprayed - unsprayed)/sprayed and expressed in percentage.

2.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) using Genstat for window 17th edition (Payne et al., 2014). Mean separation was performed using the least significant difference (LSD) procedure at a 5% probability level. Spearman correlation coefficient values were calculated to determine trait associations. Separate ANOVA were conducted per location with genotypes as the main effect and later combined ANOVA were calculated across locations after homogeneity of variance tests.

2.3 Results

2.3.1 Weather conditions

Mean monthly temperatures and rainfall were recorded at each site (Table 2.2). Adet experienced lower rainfall and higher temperatures than the other two locations. Highest rainfall was encountered at Injibara, whereas the lowest temperature was recorded at Debark. This indicates that the fungal pathogen was exposed to a wide range of environments during the cropping season.

Table 2.2 Total monthly rainfall and temperatures of the sites during the study

	Total monthly rainfall (mm)						Mean monthly air temperature (°C)					
Sites	June	July	Aug.	Sept.	Oct	Total	June	July	Aug.	Sept.	Oct	Mean
Injibara	265.8	427.5	405.9	399.3	114.6	1613.1	17.5[a]	16.8	16.2	15.9	17.2	16.5
Adet	130.6	204.9	194.1	151.8	108.5	789.9	19.6	18.7	17.6	17.8	18.3	18.4
Debark	108.5	231.5	290.5	201.0	44.1	875.6	15.0	14.2	13.6	13.8	13.8	14.1

Source: Ethiopian Meteorology Agency, [a] Temperature data at Injibara obtained from personal data logger (Watchdog Data Logger, Spectrum Technologies, Plainfield, IL, USA)

2.3.2 Analyses of variance

Analyses of variance for the traits measured are presented in Table 2.3. Highly significant ($P<0.001$) differences were detected among genotypes, treatments and environments for all the traits examined. This indicates that there is variability in the genetic makeup of the clones. The sites exhibited significant differences suggesting the existence of variation among the prevailing environments. The combined analysis of variance showed highly significant ($p<0.001$) interactions of genotype x environment, genotype x treatment, treatment x environment, and genotype x treatment x environment for all the parameters measured except for treatment x environment interaction for total and marketable tubers number per plant. The significant interaction shows that the clones did not respond the same in all environments and treatments.

Table 2.3. Analysis of variance involving 24 clones at three locations during 2014 growing season

Location		Traits and mean squares							
Source of variation	d.f.	rAUDPC	AUDPC	DT_5	TTW	MTW	TTN	MTN	RYL%
Injibara									
Rep	1	0.036	1252675	252	2.55	2.26	0.215	0.239	371.7
Gen	23	0.032***	1113829***	132***	11.70***	11.70***	3.387***	2.596***	568.3***
Residual	23	0.003	91435	25	1.15	1.14	0.399	0.513	128.6
Adet									
Rep	1	0.0001	1213	3	48.23	57.65	0.267	0.519	0.73
Gen	23	0.0206***	251740***	98***	56.69***	51.41***	18.58***	11.041***	406.3***
Residual	23	0.0014	16884	19.4	12.62	10.1	3.16	1.299	37.88
Debark									
Rep	1	0.0004	4304	6.75	57.72	51.007	0.012	6.822	
Gen	23	0.0661***	764359***	154**	70.236***	63.495***	14.35***	8.865***	
Residual	23	0.0044	50457	42.6	4.461	4.414	2.932	1.344	
Combined analysis of genotype and environment in experiments under late blight pressure									
Rep (Env)	1	0.0086	345984	75.1	0.294	1.274	0.252	0.656	
Gen	23	0.0742***	1317503***	266***	51.771***	53.716***	16.24***	9.916***	
Env	2	0.2076***	20562089***	1356***	12477***	10829***	611.7***	470.06***	
Gen.Env	46	0.0222***	406213***	59**	43.425***	36.445***	10.04***	6.293***	
Residual	71	0.0031	64283	30.9	7.428	6.616	2.106	1.12	
Combined analysis of genotype, treatment and environment (Adet and Injibara)									
Rep (Env)	1				108232	131055	2.498	0.013	
Gen	23				99739***	99260***	35.17***	20.984***	
Env	1				29831368***	25885381***	1816.24***	1392.052***	
Trt	1				3707693***	3458444***	336.28***	278.348***	
Gen.Env	23				96228***	95304***	22.75***	16.616***	
Gen.Trt	23				50764***	45331***	6.68***	5.247***	
Env.Trt	1				193855***	167242***	1.641^{ns}	0.181^{ns}	
Gen.Trt.Env	23				42785***	40923***	8.19***	5.152***	
Residual	95				3908	4115	1.73	0.879	

Significance levels: ** p≤0.01; ***p≤0.001; ns = non-significant; df = degrees of freedom; AUDPC = area under the disease progress curve; rAUDPC = relative area under disease progress curve; DT_5 = days to 5% disease severity threshold; TTY = total tubers yield; MTY = marketable tubers yield; TTN = Total tubers number; MTN = marketable tubers number; RYL%= relative yield loss percentage; Rep = replication; Gen = genotype; Env = environment; Trt = treatment

2.3.3 Late blight disease severity

Late blight developed in the unsprayed plots across all three test environments. Late blight developed uniformly on the susceptible spreader row until the vines were 100% blighted. No disease was detected in the fungicide-treated plots both at Adet and Injibara sites. In general, Adet had lower rAUDPC (0.18) followed by Debark (0.24), while Injibara had the highest rAUDPC (0.31) (Table 2.4). The lower rAUDPC value at Adet could be associated with a relatively dry weather experienced during the study period (Table 2.2). The rAUDPC values for individual genotypes at the three environments varied from 0.01 (most resistant) to 0.63 (most susceptible). A comparison of rAUDPC values within locations and averaged across locations had the following ranges for the genotypes 396004.263 (0.04 - 0.13), 396038.105 (0.01 - 0.20), 396029.25 (0.02 - 0.17), 393220.54 (0.04 - 0.25) and 395011.2 (0.05 - 0.24) displaying the lowest rAUDPC. In contrast, rAUDPC was greatest on local cultivars, including Shenkola (0.31 - 0.53), Jalene (0.30 - 0.48), Guassa (0.25 - 0.45), Aba Adamu (0.27 - 0.52) as well as B3C2 clones such as 395015.6 (0.26 - 0.54) and 395112.32 (0.25 - 0.63). All of the local cultivars were in the higher half of the interval susceptibility scale among the 24 clones tested, except Gudene. Some clones were highly variable in their rAUDPC values across the three locations. For example, Belete had higher rAUDPC value of 0.42 at Adet than at Injibara (0.21) and Debark (0.07), with extreme susceptibility scale ranging from 1 (Debark) to 9 (Adet).

Table 2.4. Relative area under the disease progress curve (rAUDPC) and susceptibility scale of 24 potato genotypes evaluated at three environments under late blight disease pressure

		Injibara		Adet		Debark		mean	
	Genotype	rAUDPC	[a]Scale	rAUDPC	Scale	rAUDPC	Scale	rAUDPC	Scale
1	396004.263	0.13 k	2	0.04 jk	1	0.05 gh	1	0.07	1
2	396029.25	0.17 k	2	0.04 k	1	0.02 i	0	0.08	1
3	396038.105	0.20 i-k	3	0.01 l	0	0.04 hi	1	0.08	1
4	393220.54	0.25 d-h	3	0.14 g-j	3	0.04 hi	1	0.14	2
5	395011.2	0.24 f-j	3	0.17 c-h	4	0.05 gh	1	0.15	2
6	Gudene	0.13 k	2	0.17 c-f	4	0.18 ef	3	0.16	3
7	395096.2	0.30 c-g	4	0.14 f-i	3	0.05 h	1	0.16	3
8	395109.34	0.19 jk	3	0.08 g-j	2	0.20 de	3	0.16	3
9	395017.229	0.34 a-e	5	0.10 c-g	4	0.06 e-g	1	0.19	3
10	396034.103	0.22 e-i	3	0.21 c-f	5	0.15 e-g	2	0.19	3
11	396031.108	0.22 g-j	3	0.15 f-i	4	0.24 b-e	4	0.21	3
12	395077.12	0.31 c-g	4	0.10 g-j	2	0.22 c-e	3	0.21	3
13	Gorebella	0.36 a-e	5	0.08 jk	2	0.24 c-e	3	0.23	4
14	Belete	0.21 h-k	3	0.42 a	9	0.07 f-h	1	0.23	4
15	395017.14	0.53 ab	7	0.14 f-i	3	0.20 ef	3	0.29	5
16	396038.101	0.32 b-f	4	0.16 c-i	4	0.41a-c	6	0.29	5
17	392633.64	0.32 a-f	4	0.22 c-f	5	0.48 a-c	7	0.34	6
18	395015.6	0.54 a	7	0.26 c-f	6	0.28 a-e	4	0.36	6
19	Guassa	0.45 a-d	6	0.25 b-e	6	0.40 a-c	6	0.37	6
20	Aba Adamu	0.27 d-h	4	0.32 ab	8	0.52 ab	8	0.37	6
21	396038.107	0.43 a-d	6	0.20 c-f	5	0.52ab	8	0.38	6
22	Jalene	0.48 a-c	6	0.30 a-c	7	0.37 a-d	6	0.39	6
23	Shenkola	0.53 a	7	0.34 ab	8	0.31 a-d	5	0.39	6
24	395112.32	0.41 a-e	5	0.25 a-d	6	0.63 a	9	0.43	7
	Mean	0.31		0.18		0.24		0.24	
	CV (%)	16.4		20.4		27.7			

[a] Susceptibility scale, values were rounded to the nearest whole number; [b] rAUDPC = relative area under disease progress curve; [c] means in a column followed by the same letter(s) are not significantly different at $P < 0.05$

2.3.4 Days to 5% disease severity threshold (DT_5)

Days to 5% disease severity threshold (DT_5) was shorter at Injibara (62.5) due to the early onset of late blight followed by Debark (69.4) and Adet (73.0) (Table 2.5). The first late blight lesions at Injibara were observed approximately six weeks after planting. In contrast, at Adet the first lesions were not observed until many of the genotypes had begun to flower. Most genotypes which had the lowest rAUDPC such as clones 396004.263, 396029.25, 396038.105, 395017.229, and 395011.2, reached their 5% disease severity late whereas, Guassa, Jalene, Gorebella, Shenkola, 396038.107, 395015.6, and 395112.32 developed late blight lesion early.

Table 2.5 Days to 5% disease severity threshold and relative yield loss of 24 potato clones when evaluated across three environments

	Days to 5% disease severity (DT_5)				Relative yield loss%		
Genotype	**Injibara**	**Adet[a]**	**Debark**	**Mean**	**Injibara**	**Adet**	**Mean**
396029.25	72.0 ab	83.0 ab	90.5 a	81.8	22 gh	34 a-d	28
396004.26	75.0 a	83.0 ab	79.0 a	79	58 a-f	8 i	33
395011.2	67.5 a-c	78.0 a-c	82.5 a	76	53 c-f	6 i	30
396038.11	67.0 a-c	88.0 a	71.0 ab	75.3	47 d-g	39 ab	43
395017.23	68.5 ab	78.0 a-c	79.0 a	75.2	62 a-f	12 hi	37
393220.54	67.0 a-c	78.0 a-c	79.0 a	74.7	50 c-f	23 c-h	37
Belete	72.0 ab	66.0 de	85.0 a	74.3	53 c-f	33 a-d	43
395096.2	67.5 a-c	78.0 a-c	71.0 ab	72.2	53 c-f	21 d-i	37
395077.12	65.0 a-d	83.0 ab	67.5 a-c	71.8	62 a-f	27 b-g	45
Gudene	65.0 a-d	78.0 a-c	67.5 a-c	70.2	16 h	15 g-i	15
396034.1	60.5 b-f	73.5 b-d	71.0 ab	68.3	54 b-f	13 f-i	33
396031.11	67.0 a-c	69.0 c-e	67.5 a-c	67.8	51 c-f	30 b-f	40
395109.34	67.5 a-c	69.0 c-e	64.0 a-d	66.8	43 e-g	33 a-d	38
Aba Adamu	69.5 ab	67.5 c-e	60.0 de	65.7	53 c-f	16 e-i	34
392633.64	62.5 b-e	72.0 cd	60.0 b-e	64.8	51 c-f	8 i	30
395017.14	56.0 c-g	73.5 b-d	64.0 a-d	64.5	88 a	28 b-e	58
396038.1	62.5 b-e	67.5 c-e	62.0 b-e	64	43 fg	37 a-c	40
395112.32	65 a-d	67.5 c-e	58.0 e	63.5	62 a-f	16 e-i	39
Shenkola	53.5 d-g	66.0de	67.5 ab	62.3	71 a-e	7 i	39
395015.6	51.0 e-g	66.0 de	67.5a-c	61.5	81 ab	13 f-i	47
Gorebella	49.0 fg	69.0 c-e	64.0 a-d	60.7	62 c-f	40 ab	51
Jalene	48.5 g	67.5 c-e	65.5 a-c	60.5	77 a-c	45 a	61
Guassa	47.5 g	69.0 c-e	62.0 b-e	59.5	76 a-c	46 a	61
396038.11	53.5 d-g	61.0 e	60.0 c-e	58.2	75 a-d	23 c-h	49
Mean	62.5	73	69.4	68.3	57	24	40.4
CV (%)	8	6	9.4		20	26	

[a] means in a column followed by the same letter(s) are not significantly different at P=0.05

2.3.5 Yield loss

A reduction in tuber yield was experienced in all genotypes in the diseased plots compared to the sprayed plots (Table 2.5). However, there was a wide variation in the relative yield loss among environments and genotypes. Yield loss ranged from 16 to 88% at Injibara and from 6 to 46% at Adet. At Injibara, the lowest yield reduction occurred on Gudene (16%), 396029.25 (21.97%) and 395109.34 (42.59%), while the highest loss was recorded in clones 395017.14 (88%), 395015.6 (82%) and Jalene (77%). At Adet, the clones with low yield loss were recorded in the genotypes 395011.2 (6%), Shenkola (7%) and 396004.263 (8%). The genotypes Guassa, Jalene and Gorebella had the highest yield losses recorded at 46%, 45%, and 40%, respectively. Average relative yield loss percentage for two locations revealed that clones 396029.25, Gudene, 392633.64, 395011.2, 396004.263 and 396034.103 were among the most tolerant/resistant genotypes with the lowest yield loss when compared to the rest of the clones. The cultivars Guassa and Jalene were heavily infected, with yield losses estimated at 61%. Most of the genotypes that had lower rAUDPC and DT_5, showed lower yield reduction. However there are some genotypes that had lower yield levels than expected. For example clone 396038.105 exhibited lower rAUDPC value.

However it showed higher yield loss of >40% indicating the high sensitivity of the genotype to late blight disease. Conversely, genotypes 392633.64, Aba Adamu, Shenkola and 395112.32 had higher rAUDPC values (>5 susceptibility scale) and short DT_5, however with less (<40%) yield loss. This could be attributed to their tolerance to late blight infection.

2.3.6 Total tuber yield

There was significant variation in total tuber yield among the tested clones under late blight infection across the three locations (Table 2.6). Overall, the highest yield was recorded at Adet (38.8 t ha^{-1}) followed by Debark (23.9 t ha^{-1}) and Injibara (6.54 t ha^{-1}). At Injibara the clones 396034.103, Belete and 396029.25 with mean yields of 12.3, 10.4, and 10.0 t ha^{-1}, respectively were the best yielding and these clones are not significantly different in terms of total yield. At Adet, the highest yielding clones under late blight epidemics were 396038.107 (48.8 t ha^{-1}), 396038.105 (47.0 t ha^{-1}) and 395017.229 (46.6 t ha^{-1}). At Debark, the clones 396038.105 (38.2 t ha^{-1}), Belete (32.4 t ha^{-1}) and Guassa (31.5 t ha^{-1}) had the highest yields. The best yielding cultivars in each location were resistant genotypes that showed ≤4 interval susceptibility scale except clones 396038.107 (Adet) and Guassa (Injibara). This suggests high yield potential for these two clones, though both had high (>50%) average yield loss.

Table 2.6 Total tuber yield of 24 potato genotypes under late-blight pressure when evaluated at three environments in north-western Ethiopia

	Sites and total tuber yield (t ha^{-1})			
Genotypes	**Injibara[a]**	**Adet**	**Debark**	**Mean**
396038.105	8.3 b-d	47.0 ab	38.2 a	31.2
395017.229	6.8 c-g	46.6 ab	28.0 b-e	27.1
395077.12	7.5 c-e	41.6 a-e	29.0 b-d	26.0
396034.103	12.3 a	36.6 d-h	27.2 c-e	25.3
Guassa	4.8 f-j	39.5 b-g	31.5 bc	25.3
396029.25	10.0 ab	36.0 d-h	29.7 b-d	25.2
Belete	10.4 ab	30.9 h	32.4 b	24.6
396004.263	5.0 e-j	40.4 a-f	28.0 b-e	24.5
396038.101	7.1 c-f	42.6 a-d	21.9 f-h	23.8
396038.107	4.5 g-k	48.8 a	17.7 hi	23.7
395112.32	5.9 d-j	46.2 a-c	18.8 g-i	23.6
393220.54	6.8 c-g	36.2 d-h	25.8 d-f	22.9
395109.34	9.0 bc	30.3 h	29.2 b-d	22.8
392633.64	5.6 e-j	41.5 a-e	20.0 gh	22.4
Gorebella	7.3 c-f	35.7 d-h	23.6 e-g	22.2
395011.2	4.1 h-k	41.8 a-e	19.9 gh	22.0
Shenkola	3.8 i-k	37.7 c-h	23.5 e-g	21.6
395017.14	2.2 k	41.4 a-e	20.9 gh	21.5
Jalene	4.1 h-k	40.9 a-e	18.7 g-i	21.2
395096.2	6.5 d-h	37.2 d-h	19.1 g-i	20.9
Gudene	8.3 b-d	31.9 f-h	20.9 gh	20.4
396031.108	7.4 c-e	31.6 gh	18.6 g-i	19.2
Aba Adamu	6.0 d-i	34.0 d-h	14.3 i	18.1
395015.6	3.4 jk	33.7 e-h	17 hi	18.0
Mean	6.5	38.8	23.9	23.1
CV (%)	16.4	9.2	8.8	

[a] means in a column followed by the same letter(s) are not significantly different at P=0.05

2.3.7 Marketable tuber yield

Genotypes ranked the same for marketable tuber yield (MTY) and total tuber yield. The highest MTY was recorded at Adet (35.9 t ha^{-1}) followed by Debark (21.9 t ha^{-1}) and Injibara (5.8 t ha^{-1}) in a similar order like total tuber yield. The loss in marketable yield, however, is relatively higher than the total tuber yield. At Injibara, marketable yield was reduced by 61.5% due to the disease pressure. At Adet the yield loss was 26.8% (Table 2.7).

Table 2.7 Marketable tuber yield of 24 potato genotypes when evaluated at three late blight affected environments with and without chemical control.

	Unsprayed				Sprayed		
Genotypes	**Injibara[a]**	**Adet**	**Debark**	**Mean**	**Injibara**	**Adet**	**Mean**
396038.105	7.7 b-e	46.4 a	35.9 a	30.0	15.4 d-i	72.2 a	43.8
395077.12	6.6 d-g	40.0 a-d	28.3 b	25.0	19.0 b-d	56.2 b	37.6
396029.25	9.2 a-c	35.1 b-g	28.5 b	24.3	12.5 i-m	52.7 b-e	32.6
396034.103	11.2 a	35.7 b-g	25.7 b-d	24.2	25.9 a	41.7 gh	33.8
395017.229	5.6 e-j	41.7 ab	23.4 c-e	23.6	17.1 c-g	51.8 b-f	34.4
Guassa	4.0 h-k	38.4 b-e	28.4 b	23.6	19.6 bc	72.9 a	46.2
Belete	9.9 ab	28.9 g-i	30.3 b	23.0	20.7 b	42.7 f-h	31.7
396038.101	6.6 d-g	39.4 a-e	20.9 d-g	22.3	11.6 k-m	68.1 a	39.9
396004.263	4.1 g-k	37.1 b-f	23.5 c-e	21.6	11.1 k-m	41.5 gh	26.3
395011.2	3.7 i-l	40.5 a-c	19.0 e-h	21.1	7.6 n	43.1 e-h	25.4
392633.64	5.2 e-j	38.2 b-f	19.3 e-h	20.9	10.9 l-n	44.1 d-h	27.5
395017.14	1.5 l	40.0 a-d	20.5 e-g	20.7	17.9 b-f	54.4 bc	36.1
395096.2	6.0 e-i	36.7 b-f	18.1 f-h	20.3	13.1 h-l	45.6 c-h	29.4
393220.54	6.3 d-h	31.8 e-h	22.1 c-f	20.1	13.2 h-l	43.4 e-h	28.3
Jalene	3.6 i-l	39.4 a-e	17.4 f-i	20.1	17.0 c-g	68.5 a	42.8
395112.32	5.5 e-j	37.9 b-f	16.7 g-i	20.0	14.6 f-k	50.7 b-g	32.7
395109.34	8.8 b-d	23.9 i	26.8 bc	19.9	15.3 e-j	31.8 i	23.6
396038.107	4.0 h-k	38.3 b-f	17.0 g-i	19.8	16.6 c-h	42.8 f-h	29.7
Gorebella	6.7 d-g	32.1 e-h	19.0 e-h	19.3	18.4 b-e	53.3 b-d	35.9
Gudene	7.2 c-f	30.6 f-i	17.9 f-h	18.6	9.5 mn	35.9 hi	22.7
Aba Adamu	5.1 f-j	33.4 c-h	12.8 i	17.1	11.8 j-m	38.8 hi	25.3
396031.108	6.4 d-h	27.4 hi	16.7 g-i	16.9	14.2 g-l	44.8 c-h	29.5
395015.6	2.0 kl	32.4 d-h	14.7 hi	16.4	16.9 c-g	37.5 hi	27.2
Shenkola	3.2 j-l	34.9 b-h	23.1 c-e	20.4	12.9 i-m	39.1 hi	26.0
Mean	5.8	35.9	21.9	21.2	15.1	48.9	32
CV (%)	18.3	8.9	9.6		10	8.4	

[a] means in a column followed by the same letter(s) are not significantly different at P=0.05

2.3.8 Total tubers number

Under late blight infection, the highest total tubers number was recorded at Debark site at 11.2 followed by Adet (10.8) and Injibara (4.8) (Table 2.8). At Injibara the genotype 396034.103 (with 8.2 tubers per plant), 395077.12 (7.2) and Gudene (7.0) had higher total tubers number. The total tubers number was reduced by 33.3% attributed to late blight infection. At Adet, the clones with highest tuber number under late blight endemics were 395077.12 (16.8), Jalene (16.3) and Guassa (15.4). The number is reduced by 18.8% due to the disease pressure. At Debark, clones with the highest total tubers number were Belete (17.5), 396031.108 (15.8) and Guassa (14.6).

Table 2.8 Total tubers number of 24 potato genotypes with and without chemical control of late blight disease in the highlands of north-western Ethiopia

	Total tubers number						
	Unsprayed				Sprayed		
Genotypes	Injibara[a]	Adet	Debark	Mean	Injibara	Adet	Mean
395077.12	7.2 ab	16.8 a	13.7 a-e	12.6	9.4 bc	19.3 b	14.3
Guassa	5.6 de	15.4 ab	14.6 a-c	11.8	10.6 b	19.6 b	15.1
396038.105	5.1 d-f	13.3 a-c	14.0 a-d	10.8	6.8 d-h	22.3 a	14.6
396031.108	5.9 b-d	10.2 c-e	15.8 ab	10.6	7.9 c-g	14.4 d-f	11.2
Jalene	3.5 hi	16.3 a	12.0 bg	10.6	7.7 c-g	18.6 bc	13.2
396038.101	4.2 e-h	13.4 a-c	13.0 b-f	10.2	6.1 g-j	13.6 d-g	9.8
395015.6	4.9 d-h	12.8 a-c	12.6 b-g	10.1	12.7 a	14.9 de	13.8
395017.229	4.6 d-h	15.1 ab	9.4 f-j	9.7	6.9 d-g	14.7 d-f	10.8
Belete	4.6 d-h	6.7 ef	17.5 a	9.6	7.1 d-g	7.6 kl	7.4
396029.25	5.7 c-e	10.8 c-e	11.5 c-i	9.3	6.2 f-i	16.0 cd	11.1
395096.2	4.4 e-h	9.7 c-e	12.0 b-g	8.7	6.8 d-i	9.0 i-l	7.9
395112.32	5.1 d-g	9.7 c-e	11.2 c-i	8.7	7.3 c-g	15.2 de	11.2
396034.103	8.2 a	9.1 c-e	8.9 f-j	8.7	8.5 c-e	10.4 h-k	9.5
Gudene	7.0 a-c	8.2 d-f	10.6 c-j	8.6	5.8 g-j	10.9 g-j	8.4
396004.263	4.0 f-h	9.9 c-e	11.7 c-h	8.5	4.7 lj	10.9 g-j	7.8
392633.64	3.9 f-h	11.5 b-d	8.5 g-j	8.0	6.4 e-i	8.9 j-l	7.7
393220.54	4.9 d-h	8.0 d-f	10.9 c-j	7.9	5.9 g-j	11.5 g-j	8.7
Gorebella	4.3 e-h	9.2 c-e	10.0 dj	7.8	8.6 cd	15.2 de	11.9
395011.2	3.6 hi	9.7 c-e	9.7 e-j	7.7	4j	15.4 de	9.7
Aba Adamu	4.8 d-h	10.2 c-e	6.9 j	7.3	5.9 g-j	12.8 e-h	9.4
Shenkola	4.2 e-h	9.1 c-e	8.7 g-j	7.3	4.8 h-j	9.1 i-l	6.9
395017.14	2.5 i	11.4 b-d	7.7 h-j	7.2	9.3 bc	11.9 f-i	10.6
395109.34	3.8 f-i	4.8 f	10.6 c-j	6.4	6.1 g-j	7.3 l	6.7
396038.107	3.6 g-i	7.8 d-f	7.5 ij	6.3	8.2 c-f	9.4 i-l	8.8
Mean	4.8	10.8	11.2	8.9	7.2	13.3	10.3
CV (%)	13.1	16.5	15.3		12.3	9.3	

[a] means in a column followed by the same letter(s) are not significantly different at P=0.05

2.3.9 Marketable tubers number

The marketable tubers number in each location under natural late blight infestation had similar rank as the total tubers number (Table 2.9). The highest number of marketable tubers number was recorded at Debark (8.8) followed by Adet (8.6) and Injibara (3.3). At Injibara, the genotypes 396034.103 (6.3), Belete (5.2) and 395077.12 (5.1) had the highest number of marketable tubers. At Adet, the clones with highest tubers number under late blight epidemics were 395077.12 (14.2), Jalene (12.9) and 395017.229 (11.7). At Debark the genotypes Belete (14.7), 395077.12 (12.2) and Guassa (11.8) had highest total tubers number.

Table 2.9. Marketable tubers number of 24 potato genotypes with and without chemical control of late blight disease in the highlands of north-western Ethiopia

	Unsprayed				Sprayed		
Genotypes	**Injibara**	**Adet**	**Debark**	**Mean**	**Injibara**	**Adet**	**Mean**
395077.12	5 a-c	14.2 a	12.2 b	10.5	7.3 bc	18.2 a	12.7
Belete	5.2 ab	6.4 h-k	14.7 a	8.8	5.8 e-h	6.9 f-h	6.3
Guassa	3.1 d-f	11.2 b-d	11.8 bc	8.7	8.1 b	16.9 a	12.5
Jalene	2.2 e-g	12.9 ab	9.2 c-g	8.1	5.7 f-h	16 a	10.9
396038.105	3.8 b-e	10.5 b-e	9.8 b-e	8.0	5.0 g-i	16.5 a	10.7
396038.101	3.5 c-f	9.0 c-h	10.7 b-d	7.7	5.0 g-i	12.4 b	8.7
396034.103	6.3 a	8.3 e-j	8.0 d-g	7.5	7.2 b-d	9.5 c-e	8.3
395017.229	2.8 d-f	11.7 bc	7.3 e-g	7.3	5.5 f-h	11.5 b-d	8.5
396004.263	2.8 e-g	9.3 c-f	9.8 b-e	7.3	4.9 hi	8.8 ef	6.8
396029.25	3.1 d-f	9.0 c-i	9.2 c-g	7.1	4.8 hi	13.3 b	9.0
395015.6	1.8 fg	10.4 b-e	9.0 d-g	7.0	9.4 a	11.6 bc	10.5
Gudene	4.5 b-d	7.2 f-j	7.9 d-g	6.6	3.9 l	8.4 e-g	6.2
396031.108	3.8 b-e	6.3 i-k	9.4 c-f	6.5	6.2 c-g	12.2 b	9.2
393220.54	3.8 b-e	6.4 g-k	8.9 d-g	6.4	4.9 hi	9.7 c-e	7.3
395096.2	3.2 d-f	7.9 e-j	8.3 d-g	6.4	4.9 hi	8.0 e-g	6.4
392633.64	2.9 d-f	8.2 e-j	7.4 e-g	6.2	4.8 hi	6.8 f-h	5.8
395011.2	2.3 e-g	8.8 d-i	7.3 e-g	6.2	2.6 j	11.3 b-d	6.9
395112.32	3.7 b-e	6.5 g-k	8.4 d-g	6.2	6.0 d-h	11.5 b-d	8.7
Shenkola	2.8 e-g	7.9 e-j	8.1 d-g	6.2	4.2 l	8 e-g	6.1
Gorebella	2.3 e-g	7.3 f-j	7.9 e-g	5.8	6.5 c-f	11.5 b-d	9.0
Aba Adamu	2.9 d-f	9.2 c-g	4.2 h	5.5	4.8 hi	9.8 c-e	7.3
395017.14	1.1 g	8.4 e-j	6.5 gh	5.3	6.0 d-h	9.3 de	7.6
395109.34	3.2 d-f	4.0 k	8.8 d-g	5.3	5.0 g-i	5.4 h	5.2
396038.107	2.7 e-g	5.9 Jk	6.5 f-h	5.0	7.1 b-e	6.4 gh	6.7
Mean	3.3	8.6	8.8	6.9	5.6	10.8	8.2
CV (%)	21.8	13.2	13.2		9.6	9	

[a] means in a column followed by the same letter(s) are not significantly different at P=0.05

The marketable tubers number was reduced by 41.1 and 20.4% at Injibara and Adet, respectively. In general, the results revealed that late blight disease affected all the measured yield parameters at both Injibara and Adet although the disease severity differed among genotypes. The disease had significant effect on marketable and total tubers yield leading reduction of 44 and 40%, respectively. Significant, but relatively less effect on total and marketable tubers number was recorded due to the disease with reductions of 26 and 31%, in that order.

2.3.10 Relationships between yield and disease resistance parameters

Spearman's rank correlation coefficients were calculated among the AUDPC, DT_5, RYL%, TTY, MTY, TTN, and MTN to determine associations between the parameters assessed (Table 2.10). Due to the presence of clone x location interactions, the correlation analysis is presented for each location separately. Significant negative correlations were detected between AUDPC and DT_5 across the three environments ($P<0.001$). AUDPC had a negative correlation with TTY and MTY at Injibara and Debark ($P<0.001$), and with TTN and MTN at Injibara ($P<0.01$) and Debark ($P<0.05$). Conversely, non-significant correlation was observed

between AUDPC and yield and yield related traits at Adet. DT_5 had a significant and positive correlation (P<0.01) with TTY and MTY at Debark and Injibara and significant correlation (P<0.05) at Adet. Similarly, DT_5 had significant (p<0.05) and positive correlation with MTN and TTN at Debark, highly significant (p<0.01) and significant (p<0.05) correlation with MTN at Injibara and Adet, respectively. However, weak association had been detected between DT_5 and TTN at both Injibara and Adet. Total tuber yield had highly significant (p≤0.01) and positive correlation with MTY, TTN and MTN in all the environments. A positive correlation was found between relative yield loss and AUDPC at both Injibara (p<0.001) and Adet (p<0.01). At Injibara, relative yield loss had highly significant (P<0.01) and negative correlation with DT_5, TTY, MTY, TTN, and MTN. Significant and negative correlation was found between relative yield loss and TTN (p<0.01) and MTN (p<0.05) at Adet.

Table 2.10 Pair-wise correlation coefficients showing association of late blight disease and yield related parameters of 24 potato clones tested at three sites in north-western Ethiopia

Traits	**AUDPC**	**DT_5**	**TTY**	**MTY**	**TTN**	**MTN**
Injibara						
AUDPC	1.00					
DT_5	-0.78***	1.00				
TTY	-0.64***	0.39**	1.00			
MTY	-0.62***	0.39**	0.99***	1.00		
TTN	-0.34**	0.14^{ns}	0.57***	0.52***	1.00	
MTN	-0.49***	0.30**	0.72***	0.71***	0.66***	1.00
RYL%	0.78***	-0.56***	-0.73***	-0.72***	-0.36**	-0.50***
Adet						
AUDPC	1.00					
DT_5	-0.77***	1.00				
TTY	-0.11^{ns}	0.19*	1.00			
MTY	-0.12^{ns}	0.31**	0.91***	1.00		
TTN	-0.04^{ns}	0.18^{ns}	0.46***	0.63***	1.00	
MTN	-0.05^{ns}	0.25*	0.39**	0.60***	0.85***	1.00
RYL%	-0.31**	0.15^{ns}	-0.12^{ns}	-0.18^{ns}	-0.36**	-0.29*
Debark						
AUDPC	1.00					
DT_5	-0.91***	1.00				
TTY	-0.58***	0.48***	1.00			
MTY	-0.54***	0.45***	0.95***	1.00		
TTN	-0.21*	0.30**	0.37**	0.34**	1.00	
MTN	-0.24*	0.31**	0.55***	0.56***	0.84***	1.00

Significance levels: *p≤0.05; ** p≤0.01; ***p≤0.001; ns=non-significant; AUDPC = area under the disease progress curve; DT_5 = Days to 5% disease severity threshold; TTY = total tuber yield; MTY = marketable tuber yield; TTN = total tubers number; MTN= marketable tubers number; RYL%= relative yield loss percentage

2.4 Discussion

The present study evaluated the response of 24 selected potato clones for late blight disease and yield and related traits at three major potato growing locations in the highlands of north-western Ethiopia. The study included 17 clones from a B3C2 population developed by CIP

for their non-specific resistance to late blight (Landeo et al., 1995), while seven were widely grown local potato cultivars. Disease development varied across locations resulting in differential responses of genotypes for late blight severity and yield reduction. AUDPC values were the highest, DT_5 was shorter, relative yield loss was greater and the total and marketable yield were the lowest at Injibara site followed by Debark and Adet. The severity of late blight and the yield reduction seems to be correlated with the amount of precipitation received during the growing season (Table 2.2). Umaerus and Umaerus (1994) and Hannukkala et al. (2007) also explained that environment does play a considerable role in the development of late blight. Temperature and humidity are the principal factors that affect disease development. Generally, moderate temperatures (10-25 °C) and wet conditions (100% relative humidity) are required for sporulation (Harrison, 1992). The present study displayed the Injibara site which had the most favorable environment for late blight disease development, had the highest disease severity, providing the best discrimination among the tested clones. The lower coefficient of variation recorded for AUDPC and relative yield loss percentage also confirms more uniform disease development at Injibara than the other sites.

Significant genotype x location interaction was observed for late blight resistance and yield and yield related traits. The tested clones exhibited an interval susceptibility scale value differences less than 4 across the three locations, except cultivar Belete. Interestingly, cultivar Belete displayed the highest late blight susceptibility at Adet despite the relatively lower disease pressure at the site. However, this clone was amongst the most resistant genotypes with lower disease severity at both Injibara and Debark sites. The present findings contradicts the observation of Haynes et al. (1998) who reported that highly resistant and susceptible genotypes were the most stable but that some of the intermediate clones were less stable. Observation of partially resistant clones behaving differently to *Phyphthora* infection in different locations were also reported by Parker et al. (1992), Mulema et al. (2004) and Forbes et al. (2005). The discrepancies in late blight severity shown in some genotypes over the locations could be associated with isolate variability, adaptation to quantitative resistance, environmental difference and/or a combination of all (Flier et al., 2003; Forbes et al., 2005). The population of the potato late blight pathogen in Ethiopia is A1 mating type, US-1 clonal lineage plus mtDNA haplotype **I**a, which are host specific (Schiessendoppler and Molnar, 2002). Thus the interaction effect could be associated with the presence of unknown R gene in the genotype or change in pathogen genotype in the particular location. However, more detailed research would be required to test diversity of pathogen genotypes among the locations.

The same inference can be made for the rank of changes observed in yield and yield related traits of some clones. The magnitude of the yield loss differed with disease severity as it is

verified by significantly negative correlation of resistance parameters with yield and related traits in the present study. Likewise, variation in environmental factors such as daily temperature fluctuations, rainfall, soil types, etc. are critical in affecting tuber yield (Kooman et al., 1996; Khan, 2012). Thus, the differences in tuber yield and yield related parameters among the clones could be explained not only by differences in the level of disease severity but also in the yield potential of the genotypes. This can be illustrated by the genotype 395109.34 which had the highest yield at Injibara but the lowest at Adet despite its stable susceptibility scale (2-3).

Marked variability and association were detected in late blight resistance, tuber yield, marketable tuber yield, tubers number and marketable tubers number within and across locations. AUDPC was highly correlated with DT_5 in all the three study locations suggesting that susceptible cultivars succumb to the disease early resulting in higher AUDPC values. Similar findings have been reported by Dorrance et al. (2001) reporting that specific components of resistance such as infection efficiency, latent period, and lesion growth rate, which are included in the measurement of DT_5, would likely contributed to partial resistance. They also suggested that DT_5 is the most efficient method to measure components of resistance under field condition. Significant correlation was found between RYL% and AUDPC in both test locations confirming the great potential of AUDPC in detecting differences in disease development between cultivars.

A negative correlation was also found between AUDPC and yield and yield related traits, i.e., TTW, MTW, TTN and MTN under chemically unsprayed conditions at the Injibara and Debark sites. The result indicates that yield and yield related traits decrease with increased severity. At Adet, weak correlation was found between AUDPC and yield and yield related traits and between RYL and DT_5, TTY and MTY. This could be associated with lower severity and the late appearance of the disease at Adet. Mean rAUDPC values were approximately two fold higher at Injibara than in Adet. Estimation of low levels of disease severity often leads to high standard errors because of irregular distribution of disease within the crop (Danielsen and Munk, 2004). In the presence of high disease severity at Injibara, RYL% was highly and negatively correlated with DT_5 and yield and yield related parameters as expected. The strong correlation between RYL% and DT_5 indicates that the early appearance of the disease has greatest potential to cause serious yield reductions.

Significant variation was observed among the clones with regard to late blight infection under sprayed and unsprayed regime (Figure 2.1). The best eight genotypes with the highest late blight resistance (rated with interval susceptibility scale of ≤3, longer DT_5 and ≤37% yield loss) and stability across the three locations were 396004.263, 396029.25, 393220.54,

395011.2, Gudene, 395096.2, 395017.229 and 396034.103. Clones 395109.34, 396031.108 and 395077.12 had moderate resistance (interval susceptibility scale of 3 and intermediate yield loss ranging from 37 to 50%). Many of these clones are therefore potential parents for late blight resistance breeding programs. Among these 396029.25, 395017.229, 396004.263 and 396034.103 and 395077.12 were relatively high yielding (≥ 25 t ha^{-1}), all of which are clones selected from B3C2 population developed by CIP. The present findings demonstrate that high levels of resistance are available in the B3C2 population. The candidate clones exhibiting adequate levels of late blight resistance and with high yields can be valuable genetic resources for breeding programs and/or for large scale production after yield stability tests. Breeding gains in resistance and minimum yield loss could be achieved through selection and recombination using these genetic stocks. The most susceptible genotypes across the study sites (> 4 interval susceptibility scale and >50% yield loss) were 396038.107, Guassa, 395015.6, 396038.101, 395017.14 and Gorebella. The present study showed that late blight resistance levels of the B3C2 clones were more variable under the present environments than their 'resistant to moderately resistant' reaction reported by CIP (Table 2.1). This could be attributed to differences in pathotypes of the disease and the environments. The same result has also been found by Yao et al. (2011).

Figure 2.1 Late blight susceptible genotype 395017.14 with chemical spray (A) and unsprayed condition (B); resistant genotype 395011.2 when chemical sprayed (C) and unsprayed (D) at Injibara site of the north-western Ethiopia during the 2014 main cropping season.

2.5 Conclusions

Results from the current study revealed significant differences in the level of resistance to late blight disease and yield and related traits among the tested potato clones. The following clones: 396004.263, 396029.25, 393220.54, 395011.2, Gudene, 395096.2, 395017.229, 396034.103, 395109.34, 396031.108 and 395077.12 had resistant to moderately resistant reaction to late blight disease across the study locations. All the local cultivars except Gudene were susceptible to late blight suggesting the need for strategic resistance breeding using the novel parents. Correlations between AUDPC, DT_5 and RYL were significantly positive indicating that early appearance of the disease could result higher AUDPC values and yield loss. Strong and significant correlation existed between AUDPC and DT_5 across the study sites suggesting that DT_5 was the most important parameter in identifying the resistant clones. Overall the study identified high yielding clones with adequate level of late blight resistance which are recommended for breeding or direct production after yield stability tests.

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CHAPTER 3. Combining ability of selected potato clones for resistance to late blight disease, yield and yield components

Abstract

Late blight of potato caused by *Phytophthora infestans* is a most destructive disease. Breeding potato cultivars with resistance to late blight is the most economic, effective and ecologically sustainable method to control the disease and to boost productivity. This study was, therefore, carried out to determine combining ability and gene action controlling late blight resistance, yield and yield components and to identify promising potato genotypes as potential parents in a breeding programme. Eighteen F_1 families were generated from two sets of 12 parents using a North Carolina Design II. The families were evaluated for relative area under disease progress curve (rAUDPC), yield and yield related traits in two hotspot locations for late blight. Results showed that the general combining ability (GCA) effects of female and male parents, and the specific combining ability (SCA) of families were significant for all the traits except for the GCA effect of female for marketable tuber yield. Estimates of genetic components indicated that additive component of genetic variance was more important than the non-additive component in inheritance of resistance to late blight (rAUDPC= 71%) and average tuber weight (80%). The GCA and SCA effects were almost equally important for the total and marketable tuber yields [GCA/ (GCA+SCA) = 53%]. Among the parents: 396264.14 and 395109.34 were selected for their good GCA effects for both late blight resistance and yield related traits, while the parent 396004.263 had strong ability to transfer late blight resistance to its progenies. Crosses from 396004.263 x 395017.229 and 395096.2 x 396012.288 were best combiners displaying significant SCA effect for both late blight resistance and yield related traits in the desired direction and the cross from 395109.7 x 396264.14 had negative and significant SCA effect for late blight resistance as measured by rAUDPC. The selected parents and families were the best candidates to develop improved potato cultivars that combined both high yield and adequate late blight resistance.

Key words: AUDPC, breeding, Ethiopia, North Carolina Design II, tetraploid potato

Late blight of potato caused by *Phytophthora infestans* (Mont.) de Bary is the most devastating disease in the humid tropical highlands of Ethiopia (Mulatu et al., 2005; Hirpa et al., 2010). The disease causes an estimated yield losses ranging between 30 and 100% in different parts of the country (Kassa and Beyene, 2001; Mulatu et al., 2005). Several control measures can be employed against late blight disease. Given the fact that potato is mostly grown by resource poor farmers, the most economic and environmentally sound control

measure could be the use of resistant cultivars (Colon et al., 1995; Forbes, 2012). Although breeding for late blight resistance has had priority in potato breeding program in Ethiopia for more than three decades (Hirpa et al., 2010), many resistant cultivars thus far released have quickly succumbed to virulent races of the pathogen (Woldegiorgis, 2013).

Resistance to *P. infestans* are classified into race-specific and race-nonspecific resistance. Presently resistance breeding focused on polygenically controlled, quantitative resistance which may substantially reduce levels of disease. This form of resistance is more durable as it is effective against a broad range of pathogenic races of the disease (Umaerus and Umaerus, 1994). Conversely, race specific resistance controlled by major resistance (R) gene can be readily overcome by rapid appearance of virulent races of the pathogen (Fry, 1977; Haynes et al., 1998). Based on this concept, the international potato centre (CIP) developed advanced population group B3 with quantitative resistance which are free of any known major (R) genes by recurrent selection. Late blight resistance of population B3 has been improved after every cycle of recombination (B3C0, B3C1 and B3C2) (Landeo et al., 2001; Kaila, 2015). Also, the population have wider genetic background of various economic traits. The germplasm are available as source of breeding population for durable late blight resistance in potatoes (Landeo et al., 1995; Landeo et al., 2001; Yao et al., 2011). Some of the advanced clones from this population were evaluated in Ethiopia and showed promising performance (CIP, 2012).

For a successful potato breeding program for late blight resistance, it is necessary to select parental clones capable of transmitting the resistance to their offspring. Parental genotypes which transmit superior economic traits to their offspring when crossed with a wide variety of other clones are said to have good general combining ability (GCA). The deviation of a specific cross from what is expected on the basis of the GCA of the parent is referred to as specific combining ability (SCA) (Plaisted et al., 1962; Gopal, 1998). Genetically, GCA is associated with genes which are additive in their effects. SCA, on the other hand, caused by dominance and epistasis gene effects (Falconer and Mackay, 1996; Acquaah, 2007). Information on the components of general and specific combining ability are helpful to select promising parents and novel genetic combinations for effective breeding. When GCA effects are significant, parents with desirable effects can be used for future crosses which may yield improved selection gain. When SCA effects is present, the progeny tests can be used to identify the best crosses for clonal evaluation and cultivar release (Gopal, 2015).

Use of appropriate mating design is a key factor for proper estimation of combining ability effects. Different mating designs have been used to study the genetic determination of various traits of potato. For example: Killick and Malcolmson (1973) used a North Carolina

Design II and reported that the SCA was more important than GCA effect for late blight resistance. Malcolmson and Killick (1980) found significant GCA effect using the same design. Plaisted et al. (1962) and Kaushik et al. (2000) found larger estimate of SCA effect than the GCA effect for yield and late blight resistance, respectively using a line x tester genetic design. Kumar et al. (2007), on the other hand, reported the predominance of additive component of genetic variance over the non-additive component using line x tester genetic design. Other researchers used incomplete diallel (Tai and Hodgson, 1975) and full diallel (Bradshaw et al., 1995) designs to determine combining ability effects of different traits.

North Carolina Design II (NCD II or factorial) provides good genetic information on the reference population for the trait(s) being investigated (Hallauer et al., 1988; Ortiz and Golmirzaie, 2002). Advantages of a factorial design includes: addition of more parents without a significant increase in resources and estimation of additive variances of males and females (Lynch and Walsh, 1998).

In Ethiopia, potato improvement has been limited to evaluation of CIP and local genotypes (CIP, 2012; Woldegiorgis, 2013). No information is available in Ethiopia on the combining ability and the gene action controlling resistance of potato genotypes against the present pathotypes of *P. infestans* and their yield performance. There is a need for a well-designed breeding program in the country to improve late blight resistance, yield and yield related traits. Clonal selection and information on combining ability for the desired traits among selected potato genotypes is important in order to identify the best combiners for successful breeding. The objective of the present study was therefore to determine the combining ability and gene action controlling late blight resistance, yield and yield components and to identify promising parents and crosses based on their combining ability effects among selected potato cones of B3C2 population for further breeding and selection in north-western Ethiopia.

3.1 Materials and methods

3.1.1 Parents and crosses

Twelve clones from the B3C2 population showing variable late blight resistance were crossed using a North Carolina Design II (NCD II) (Table 3.1). Crosses were performed in two sets, six parents each to generate 18 families. In the first set three female clones (395015.6, 395109.34 and 396004.263) were crossed with three males (395011.2, 395017.229 and 396038.107), whereas in the second set three female parents (395096.2, 395109.7 and 396031.108) were crossed with three males (395017.14, 396012.288 and 396264.14). In total, 18 families were constituted (2 sets x 9 family each).

Table 3.1 Name of potato parents used in the study with their late blight reaction and yield level

Set	Male/Female	Parents	AUDPC-CIP	AUDPC-NWE	Yield in late blight epidemics (t ha-1)
1	Female	395015.6	1207	1670	18.03
1	Female	395109.34	690	710	22.83
1	Female	396004.263	858	355	24.46
1	Male	395011.2	703	722	21.95
1	Male	396038.107	1094	1660	23.65
1	Male	395017.229	937	934	27.13
2	Female	396031.108	724	889	19.22
2	Female	395096.2	1210	795	20.91
2	Female	395109.7	1212	-	17.66*
2	Male	395017.14	1025	1430	21.47
2	Male	396012.288	971	-	25.32*
2	Male	396264.14	389	-	21.26*

AUDPC = area under disease progress curve; CIP= International Potato Centre; NWE = north-west Ethiopia; * = yield information for the three clones obtained from CIP

3.1.2 Seedling generation

A maximum of 200 F1 seeds per cross was sown in seedling trays filled with 1:2:1 mixture of sand, farmyard manure and soil, respectively at Adet agricultural research centre. The substrate was sterilized in an oven. After 35 days, 80 to 120 seedlings of each cross were transplanted into 10 cm plastic pots for further growth. Fertilizer was applied as per recommended and pesticides were sprayed when required.

True potato seedlings from 18 crosses having 80 genetically unique siblings each harvested separately, approximately three months after transplanting. As the tubers were harvested from each plant, they were left in the pots in which the plant was grown and one tuber from each pot was taken to produce tuber families comprised of a single tuber from each true seedling (Figure 3.1). Two sets of tuber families which were selected were saved for planting in two environments. Seed potatoes were stored at Injibara using the diffused light storage facility approximately for five months prior to field evaluation.

Figure 3.1 Tubers derived from true seeds (left) and a tuber family consisting of single tuber from each seedling with in a family (right)

3.1.3 Trial establishment and experimental design

Eighteen families each having eighty progeny were planted at the two locations in north-western Ethiopia: Adet (11°17′ N, 37°47′ E and altitude 2240 m) and Injibara (10°57′ N, 36°56′ E and altitude 2568 m,), during the main crop season (June, 2014). These location are known for experiencing severe late blight disease pressure during the rainy season. Consequently, clones were evaluated under natural disease development. A susceptible local cultivar, 'Enatbeguaro' was planted adjacent to each row to increase the inoculum level and ensure infection across the plots. No control measure was taken against late blight. Trials were established using a randomized complete block design with two replications. Each entry was represented by an experimental unit consisting of forty plants assigned in a plot of 9 m^2. The plot size was four rows, 3 m long, with 0.75 m inter- and 0.3 m intra-row spacing. All the necessary agronomic practices were carried out according to the recommendations to the locations.

3.1.4 Data collection

Data collected included percentage of foliage affected by late blight disease, total tuber yield (TTY), marketable tuber yield (MTY) and average tuber weight (ATW). Late blight disease severity was recorded visually as percentage of foliage affected at weekly intervals starting with the first appearance of the symptoms until the susceptible control had reached 100% infection. The percentage foliage covered with late blight in each plant was estimated (Fry, 1978; Niks et al., 2011) and averaged per plot basis. For each plot and assessment dates, AUDPC was calculated using the method of Campbell and Madden (1990). AUDPC was standardized to estimate a relative area under the disease progress curve (rAUDPC) by dividing the AUDPC by the maximum potential AUDPC of that location (Fry, 1978).

At harvest, yield was measured in a plot basis. Total tuber yield (TTY) was calculated by converting the total weight of all the tubers harvested in a plot to t ha^{-1}. Tubers of each plot were graded in to three categories: >30 mm (marketable), <30mm (unmarketable), and rotten and diseased (discarded) and were counted and weighted in kg. Form these, the marketable tuber yield (t ha^{-1}), number of total tuber per plant and number of marketable tuber per plant were calculated. Average tuber weight (ATW) was calculated as the total tuber weight per plant divided by the total tuber number of tubers per plant.

3.1.5 Data analyses

The data for rAUDPC, total tuber yield, marketable tuber yield and average tuber weight of the two locations were subjected to the standard analysis of variance using the GLM procedure of SAS 9.3 (SAS Institute Inc, 2011) statistical program. The data were first analysed separately. After homogeneity of variance tests a combined analysis of variance was performed.

Analysis of variance was performed using the North Carolina Design II (Comstock and Robinson, 1952) with SAS version 9.3 (SAS Institute Inc, 2011) to identify the significance level of general combining ability (GCA) of parents and specific combining ability (SCA) of crosses. Data were analysed over sets and across environment using the following linear model (Hallauer et al., 1988):

$$Y_{ijkpq} = \mu + S_p + g_i(S_p) + g_j(S_p) + h_{ij}(S_p) + E_q + r_k(SE)_{pq} + (ES)_{pq} + (E_g)_{iq}(S_p) + (E_g)_{jp}(S_p) + (E_h)_{ijq}(S_p) + (E_h)_{ijq}(S_p) + e_{ijkpq}$$

Where: i = 1, 2, 3; j = 1, 2, 3; k = 1, 2; p = 1, 2; q = 1, 2; the terms for the model are defined as follows: Y_{ijkpq} denotes the value of a family from the mating between the i^{th} female parent, the j^{th} male parent, in the k^{th} replication, within set p and in the q^{th} environment; μ = Grand mean; S_p = the average effect of the p^{th} set; $g_i(S_p)$ = the GCA effect common to all F1 families of the i^{th} female parent nested within p^{th} set; $g_j(S_p)$ = the GCA effect common to all F1 families of the j^{th} male parent nested within p^{th} set; $h_{ij}(S_p)$ = the SCA effect specific to F1 families of the i^{th} female and j^{th} male parent nested within p^{th} set; E_q = average effect of q^{th} environment; $r_k(SE)_{pq}$ = the effect of the k^{th} replication nested within the p^{th} set and q^{th} environment; $(ES)_{pq}$ = the interaction between site and set effects; $(E_g)_{iq}(S_p)$ and $(E_g)_{jp}(S_p)$ = the interaction between site and GCA of the ith female and jth male parent, respectively nested within sets; $(E_h)_{ijq}(S_p)$ = the interaction between site and SCA, nested within sets; and e_{ijkpq} = the random experimental error.

Throughout the text, variation due to males within sets, females within sets, and males x females within sets were referred to as GCA_m, GCA_f and SCA variation, respectively. For each trait, GCA effect for each clone and SCA effect for each F1 family were calculated according to Beil and Atkins (1967). For rAUDPC negative estimate of combining ability (GCA and SCA) effects were taken as high in a desirable direction, while neutral or positive estimate were regarded as low or undesirable. For TTY, MTY, and ATW positive estimate of GCA and SCA effects were taken as high to identify genotypes with high yield and yield components, whereas neutral or negative estimates as low. Standard errors for GCA effects of female and male parents and SCA effects of families in each sets were calculated by using the method described by Cox and Frey (1984). The spearman correlations were calculated for selected traits and locations to study the interrelationships between these traits and the two environments. The relative importance of additive (GCA) and non-additive (SCA) genetic effects in determining the performance of the progeny for each of the traits was determined by individually expressing the GCA_f mean square, GCA_m mean square, and the SCA mean square as a percentage of the treatment (crosses) mean square as shown in the formula below (Baker, 1978):

$$GCA/ (GCA+SCA) (\%) = MS\ GCA_{Pooled}/ (MS\ GCA_{Pooled} + MS\ SCA)$$

$$MS\ GCA_{pooled} = s(f-1)MS\ GCA_f + s(m-1)MS\ GCA_m/ (s(m+f-2))$$

Where; MS GCA_{Pooled} = mean squares for GCA_{pooled}; MS SCA = mean squares for SCA effects; s = number of sets; f = number of female parents; m = number of male parents; MS GCA_f = mean square of GCA_f; MS GCA_m = mean square of GCA_m, respectively.

3.2 Results

3.2.1 Environmental Effects

Late blight disease pressure varied across the two locations (Table 3.2). Large environmental main effects were a reflection of differences in the magnitude of disease severity and yield related traits at the two locations. The mean rAUDPC at Adet and Injibara was 0.17 and 0.28, respectively. The mean temperature was lower and total precipitation was higher at Injibara during the growing season (16.5^{o}C and 1613.1mm, respectively from June to October, 2014) than at Adet (18.4^{o}C and 789.9mm, respectively). Because of more conducive environmental condition late blight disease started early at Injibara than at Adet.

3.2.2 Combining ability

The analyses of variance of rAUDPC, total tuber yield, marketable tuber yield and average tuber weight for North Carolina Design II combined across environments and sets is presented in Table 3.2. Analysis of variance pooled over sets and across environments

showed that the GCA mean square for males (GCA_m), GCA for females (GCA_f) and SCA were all significant for all the traits tested except the GCA_f effects for marketable tuber yield. The parents and the F1 families, therefore, differed significantly in their GCA and SCA, respectively for the four traits. The interactions of GCA_f x environments and of SCA x environments were only significant for average tuber yield. Interaction of GCA_m x environment was significant ($P < 0.01$) for total and marketable tuber yield. The sets are significantly different for rAUDPC ($P<0.05$) and average tuber weight ($P<0.01$). There was no significant interaction between environment and sets for all the traits assessed.

Table 3.2. Summary mean squares and significant tests of combining ability effects for rAUDPC, TTY, MTY and ATW of potato clones evaluated at two sited in north-western Ethiopia

Source of variation	d.f.	rAUDPC	TTY	MTY	ATW
Environment	1	0.2226***	22531.5***	20610.6***	35594.06***
Set	1	0.0066*	0.01502^{ns}	0.06698^{ns}	193.6787**
Environment x Set	1	0.0035^{ns}	0.72605^{ns}	0.37154^{ns}	5.24117^{ns}
Replication (Set) (Environment)	4	0.0032^{ns}	36.929***	41.3038***	111.2662**
GCA_f	4	0.0219***	14.795*	11.2265^{ns}	504.7382***
GCA_m	4	0.0111***	39.101***	42.7027***	180.8366***
SCA	8	0.0069***	24.158***	23.4957**	87.73194***
Environment x GCA_f	4	0.0024^{ns}	7.6621^{ns}	4.73641^{ns}	79.0173**
Environment x GCA_m	4	0.0028^{ns}	21.588**	25.52429**	47.26075^{ns}
Environment x SCA	8	0.0018^{ns}	7.14989^{ns}	7.55228^{ns}	48.42515*
Error	32	0.00138	4.563	5.731	19.03
GCA/ (GCA+SCA) (%)		71	53	53	80
Contribution of GCA_f (%)		47	14	11	59
Contribution of GCA_m (%)		24	42	38	21

***, **,*, ns = significant at $P< 0.001$, $P< 0.01$, $P< 0.05$ and non-significant at $P> 0.05$, respectively; d.f. = degree of freedom; rAUDPC = relative area under disease progress curve; TTY = total tuber yield; MTY = marketable tuber yield; ATW= average tuber weight; GCA_f = general combining ability due to female; GCA_m = general combining ability due to male; SCA = specific combining ability

There was a highly significant and positive correlation ($r = 0.52$, $P < 0.001$) between the locations for rAUDPC indicating that the environments x family interaction was not of the crossover type. All yield related traits had significant ($P < 0.001$) and negative($r > -0.70$) correlation coefficient with rAUDPC (Table 3.3). These results were expected because of the influence of the disease on tuber yield. The yield components also showed strong correlation to each other. Interactions of environments with the genetic components were not significant for most of the traits. Hence, results are presented pooled by environment.

Total GCA (i.e., male plus female main effects) accounted for 71 and 80% for rAUDPC and average tuber weight, and 53% for both marketable and total tuber yield, respectively. For

rAUDPC, the GCA_f effects were 1.9 times larger than GCA_m effects and the GCA_f sum of squares contributed more (47% of total variance) than the GCA_m sum of squares (24% of total variance). Similarly, for average tuber weight, GCA_f mean square was 2.8 times larger than GCA_m. GCA_f contributed 59% and GCA_m 21% for the treatment mean square of average tuber weight. In contrast, GCA_m effects were 3 times larger than GCA_f effects for total tuber yield. For this trait, GCA_m contributed 42% and GCA_f 14% for the cross sum of square. Also, for marketable tuber yield GCA_m was 3.5 times larger than GCA_f. Here, GCA_m contributed 38% and GCA_f 11% for the treatment sum of square.

Table 3.3 Pair-wise correlation coefficients of late blight disease and yield related parameters in 18 F_1 potato families tested at two locations in north-western Ethiopia

Traits	rAUDPC	Total tuber yield	Marketable tuber yield
Total tube yield	-0.72***	-	
Marketable tuber yield	-0.73***	0.99***	-
Average tube weight	-0.70***	0.88***	0.89***

*** = significant at P< 0.001; rAUDPC= relative area under disease progress curve

3.2.3 General combining ability effects and mean response of parents

Estimates of the GCA effects for the 12 parents are shown in Table 3.4. The GCA estimates for rAUDPC ranged from -0.047 for clone 396004.263 to 0.064 for 395015.6. The parents which possessed good GCA for late blight resistance were 396004.263 and 395096.2 among female and 396264.14 among male parents, which had significant and negative estimates. Clones 395015.6 and 395109.7 possessed the undesired GCA effects for late blight disease resistance as measured by rAUDPC.

Among the males tested in this study, clone 396264.14 was a good general combiner for all the characters assessed, while among females clone 395109.34 had good parental value measured as high GCA for average tuber weight, total and marketable tuber yield under the disease pressure. Clone 395109.7 also contributed favourably to increase in the average tuber weight despite its poor GCA effect for rAUDPC.

The genotypes 396012.288 and 395015.6 are associated with undesired GCA effect for both resistance and yield traits, while clone 395017.14 was a poor general combiner for average tuber weight, total and marketable tuber yield. Genotype 395017.229 was a poor combiner for total and marketable tuber yield, although it had positive effect for average tuber weight among the males. Among the parents that showed significant desirable effect for rAUDPC were 396004.263 and 395096.2 associated with negative (undesirable) effect for average tuber weight, total and marketable tuber yield.

Table 3.4 Estimates of general combining ability (GCA) effects and mean performance for rAUDPC, TTY, MYY and ATW of 18 F1 potato families evaluated in two sets across two locations of north-western Ethiopia.

Parents	rAUDPC		TTY (t ha^{-1})		MTY(t ha^{-1})		ATW (g)	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
				Set I				
Female								
395015.6	0.29	0.064**	20.8	-0.82	19.4	-0.70	39.3	-4.73**
395109.34	0.21	-0.017	23.3	1.69**	21.7	1.56	53.0	8.93*
396004.263	0.18	-0.047**	20.8	-0.88	19.2	-0.86	39.8	-4.2**
Male								
395011.2	0.25	0.022	22.3	0.67	20.8	0.70	42.5	-1.51
395017.229	0.23	-0.001	20.0	-1.64**	18.4	-1.74**	46.4	2.33
396038.107	0.21	-0.021	22.6	0.97	21.1	1.04	43.2	-0.82
SE		0.010		0.597		0.597		1.14
				Set II				
Female								
395096.2	0.20	-0.015*	21.6	-0.11	20.0	-0.19	41.9	-5.46**
395109.7	0.23	0.022**	22.3	0.61	20.4	0.20	51.4	4.12**
396031.108	0.20	-0.007	21.2	-0.5	20.2	-0.01	48.7	1.34
Male								
395017.14	0.21	-0.003	20.5	-1.21*	19.0	-1.12*	43.8	-3.52**
396012.288	0.25	0.039**	20.4	-1.24*	18.8	-1.41**	45.0	-2.32*
396264.14	0.18	-0.036**	24.1	2.44**	22.7	2.53**	53.2	5.84**
SE		0.007		0.53		0.53		0.898

SE = standard error; *, ** significantly different from zero at ≥ 1.96SE and 2.56SE respectively; rAUDPC = relative area under disease progress curve; TTY = total tuber yield; MTY = marketable tuber yield; ATW= average tuber weight

3.2.4 Specific combining ability effects and mean response of families

Families from the crosses of 395109.7 x 396264.14 and 396031.108 x 395017.14 showed significant and desirable SCA effects for rAUDPC and high SCA effects for average tuber weight, total and marketable tuber yields in the desired direction (Table 3.5). These two families had also the lowest mean rAUDPC and the highest mean TTY, MTY and ATW among the families tested. Cross 396004.263 x 395017.229 exhibited the highest and significant SCA effect in a desired direction and the highest mean for rAUDPC. However, this cross showed the lowest mean and undesirable SCA effect for yield traits.

Cross 395109.34 x 395011.2 showed the highest mean for all yield traits while crosses from 395096.2 x 396012.288 had significant and positive SCA effects and highest mean for total and marketable tuber yield. The latter cross also showed significant SCA effect in the desired direction for rAUDPC although it scored medium for this trait. Besides cross 395109.7 x

396264.14, families of 395109.34 x 395017.229, 395109.34 x 396038.107 and 395109.34 x 395011.2 were the best three in terms of average tuber yield. Most of the crosses with high mean for yield traits involved the parent 395109.34 that showed the highest GCA effect for these traits (Table 3.5).

Table 3.5 Estimates of specific combining ability effects (SCA) and mean performance for rAUDPC, TTY, MTY and ATW of potato families evaluated in two sets across two locations of north-western Ethiopia

	rAUDPC		TTY		MTY		ATY	
Sets and crosses	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA
Set I								
395015.6 x 395011.2	0.31	-0.01	20.2	-1.31*	19.1	-1.04	33.6	-4.20**
395015.6 x 395017.229	0.33	0.038*	19.4	0.16	17.4	-0.26	43.9	2.27*
395015.6 x 396038.107	0.25	-0.028*	23.0	1.15	21.7	1.31*	40.4	1.93
395109.34 x 395011.2	0.23	-0.004	24.4	0.36	22.7	0.29	50.4	-1.01
395109.34 x 395017.229	0.22	0.006	22.5	0.84	20.6	0.67	54.9	-0.41
395109.34 x 396038.107	0.19	-0.002	23.1	-1.20*	21.7	-0.97	53.6	1.43
396004.263 x 395011.2	0.22	0.014	22.4	0.95	20.7	0.75	43.5	5.21**
396004.263 x 395017.229	0.14	-0.044**	18.1	-1.00	17.1	-0.41	40.3	-1.86
396004.263 x 396038.107	0.19	0.03	21.8	0.05	19.9	-0.34	35.7	-3.36**
SE		0.014		0.597		0.597		1.14
Set II								
395096.2 x 395017.14	0.21	0.021**	18.8	-1.61*	17.4	-1.46	33.6	-4.77**
395096.2 x 396012.288	0.18	-0.052*	24.2	3.83**	22.3	3.77*	43.6	4.09**
395096.2 x 396264.14	0.19	0.031**	21.8	-2.22**	20.2	-2.3**	48.4	0.68
395109.7 x 395017.14	0.25	0.016*	20.7	-0.34	18.6	-0.66	49.5	1.56
395109.7 x 396012.288	0.29	0.021**	18.7	-2.33**	16.8	-2.13**	44.2	-4.87**
395109.7 x 396264.14	0.16	-0.036**	27.4	2.66**	25.7	2.79**	60.6	3.31**
396031.108 x 395017.14	0.16	-0.037**	21.9	1.95*	21.2	2.13**	48.3	3.21*
396031.108 x 396012.288	0.27	0.031**	18.4	-1.50*	17.1	-1.64*	47.1	0.78
396031.108 x 396264.14	0.17	0.006	23.2	-0.44	22.2	-0.49	50.5	-3.99**
SE		0.007		0.75		0.75		1.27

SE = standard error; *, ** significantly different from zero at ≥ 1.96SE and 2.56SE respectively; rAUDPC = relative area under disease progress curve; TTY = total tuber yield; MTY = marketable tuber yield; ATW= average tuber weight

3.3 Discussion

Knowledge of combining ability of clones and genetic mechanisms controlling late blight disease and yield and yield related traits are essential in the designing of a potato breeding program. These information will aid for in the selection of clones with improved late blight resistance and yield. The present study investigated combining ability effects and the mode of gene action conditioning the inheritance of late blight resistance and yield components and

identified clones for breeding under the prevailing environment of north-western Ethiopian highlands. In the present study, a total of 18 families obtained from 12 parents of wider genetic background from improved population B3C2 from CIP, were used (Table 3.1).

Significant differences among GCA_f, GCA_m and SCA effects of clones indicated the presence of sufficient genetic variability for breeding. Both additive and non-additive gene actions were important in inheritance of the traits measured (Table 3.2). However, GCA variances were of higher magnitude than SCA variances for rAUDPC suggesting that the inheritance of this trait is under the control of additive genetic effect, hence further genetic gains can be achieved by selecting superior clones. Role of additive gene effects was also predominant for average tuber weight. These results agreed with previous studies (Tai and Hodgson, 1975; Malcolmson and Killick, 1980; Bradshaw et al., 1995; Landeo et al., 1995; Landeo et al., 2001; Kumar et al., 2007) who reported that GCA was more important than SCA for late blight resistance. Landeo et al. (2001) reported large additive genetic variance for late blight resistance in a random sample of B3C1 clones using three different mating designs. Higher SCA variances than GCA variances for late blight resistance were also reported by some authors (Killick and Malcolmson, 1973; Kaushik et al., 2000). The variation in the proportions of GCA and SCA variances in various studies could be attributed to differences in genetic material used. In the present study the preponderance of additive effect for late blight resistance could confirm the absences of major (R) genes in the parents and indicated minor genes were responsible for the resistance. Where GCA predominates, progeny performance can be reliably predicted from the performance of the parents. Killick and Malcolmson (1973) suggested that traits subjected to stabilizing selection are expected to show great additive genetic variation but little degree of dominance and epistasis, whereas the reverse is true for traits subjected to directional selection that may lead narrow genetic base.

For total and marketable tuber yield, the GCA effects were only slightly more important than the SCA effects (53% for both traits) (Table 3.2). Bradshaw and Mackay (1994) concluded in their review that both GCA and SCA effects contribute to the genetic variation observed in a population. Ruiz de Galarreta et al. (2006) and Gopal (1998) reported significant SCA effects for yield. However, host plant resistance to late blight can also influence GCA and SCA estimates. Landeo et al. (2001) suggested that quantifying the yield potential and its genetic control under late blight disease pressure wouldn't reflect the actual gene frequency and its magnitude in the clones. Holland and Munkvold (2001) pointed out that yield under disease stress and yield in the absence of substantial disease stress can be considered distinct traits that may be under the control of different sets of genes. However, Carson and Wicks III (1989) suggested that selection for plant genotypes that yield well under disease stress could

be advantageous approach to select for yield potential and disease resistance simultaneously.

The magnitude of the GCA variance for female was much larger than the GCA variance for males for rAUDPC (1.9 times higher) and average tuber weight (2.8 times) (Table 3.2). Higher contribution of female parents over male parents could be associated with influence of cytoplasm for late blight resistance and tuber size. This findings, however, should be interpreted with care, because the reciprocal difference was not tested. In their studies on genetic divergence in potato, Gaur et al. (1978) discovered that the distances of the tuberosum-andigena hybrids appeared to be influenced by the cytoplasm carried by their parents. In contrast, for total and marketable tuber yield GCA_m effects were 3 and 3.5 times larger than GCA_f, respectively.

This study identified the female parent 396004.263 and the male parent 396264.14 as good general combiners for late blight resistance (Table 3.4). The latter is a good general combiner for average tuber weight, total and marketable tuber yields. The resistance of the former clone, however, was associated with poor combining ability for yield needing further improvement for yield traits. Likewise, the female parent 395109.34 has good combining ability for yield traits with appreciable resistance to late blight. By contrast, the use of clones 396012.288 and 395015.6 as parents should be avoided under the prevalence of the disease.

Crosses involving 396004.263 x 395017.229, 395096.2 x 396012.288 and 395109.7 x 396264.14 showed significantly negative SCA effect for rAUDPC suggesting that they would produce the most highly resistant progenies (Table 3.5). High family mean for the traits assessed involved at least one parent with high GCA effect in the desirable direction. For example, the family 395109.7 x 396264.14 which had the lowest mean for rAUDPC emerged from low (positive) x high (negative) GCA combination of the parents 395109.7 and 396264.14, while the cross 396004.263 x 395017.229 emerged from high x neutral GCA combinations of 396004.263 and 395017.229 for rAUDPC, respectively. Similarly, the family from the crosses of 395109.34 x 395011.2 which had the highest mean for all yield traits had high x neutral to negative GCA combination of the female and male parents for yield related traits, respectively. The family from the cross 395096.2 x 396012.288 which showed the highest mean and significant SCA effect for yield and yield traits involved female parent 395096.2 with negative GCA for rAUDPC despite both parents were from low x low GCA combinations for yield and its related traits. This result suggests that SCA effects of the families were conditioned by the GCA effects of the parents. It could also indicate that it

would be possible to develop resistant clones when crosses are made between complementary progenitors.

The non-significant interaction between environment x set and environment x genetic components for rAUDPC suggest that the performance of the families were consistent across the two tests of environment for these traits. This result was supported by significant and strong correlation for rAUDPC between the two environments.

3.4 Conclusions

The present study showed that clones 396264.14 and 395109.34 were best combiners displaying good GCA effects for both late blight resistance and yield related traits. Among the crosses 396004.263 x 395017.229 and 395096.2 x 396012.288 were selected for their best SCA effects for both late blight resistance and yield related traits. The selected parents and families were the best candidates to develop improved potato varieties with high yields and LB resistance. The predominance of variance due to GCA over SCA effects for disease resistance suggests that progeny performance can be accurately predicted based on GCA effects of the parents. Overall results indicated that it would be possible to breed for high yielding clones coupled with adequate level of late blight resistance from this sets of germplasm.

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CHAPTER 4. Combining ability of selected potato clones for drought tolerance and yield components

Abstract

Recurrent drought is one of the major impediments to potato production in the north-west Ethiopian highlands. The objectives of this study were therefore, to determine combining ability effects and gene action determining yield, yield components and drought tolerance related traits among selected potato clones and to identify promising parents and crosses for cultivar development. Sixteen selected clones were crossed in two sets using North Carolina Design II, resulting in 32 families. Families together with 17 clones were evaluated in a 7 x 7 lattice design with two replications under irrigated and managed drought stress conditions at tuber bulking stage at Adet, Ethiopia. Results showed highly significant differences among genotypes for drought stress tolerance, growth, physiological and yield related traits. Significant general combining ability (GCA) effects and specific combining ability (SCA) for all the traits assessed indicated the contribution of additive and non-additive genes in the expression of these traits, respectively. GCA effects were more important than SCA effects for total tuber yield, marketable tuber yield, average tuber weight, plant height, chlorophyll content and groundcover. This suggested the preponderance of additive gene action for these traits under drought stress. Among the parents, the clones 395112.32, 396034.103 and 396012.288 were the best general combiners for yield and drought tolerance. The families with the best specific combining ability (SCA) effects for both tuber yield and drought tolerance were 395109.34 x 396041.102, 395096.2 x 396012.288, 395109.7 x 395017.14 and 396031.108 x 395017.14. The selected parents and families are useful genetic resources to improve drought tolerance and yield of potato. The study demonstrated that high yield potential and high drought tolerance were not mutually exclusive in the tested set of germplasm.

Key words: combining ability, drought tolerance, North Carolina II mating designs, plant breeding, potatoes

4.1 Introduction

Recurrent drought is one of the most important constraints to plant growth and productivity in many regions all over the world (Chaves et al., 2002; Obidiegwu et al., 2015). In Ethiopia, drought is a frequently recurring phenomenon and its impact is magnified by threatening food security and rural livelihood. Agriculture is a major economic sector in the country, employing 85% of labour force and contributing 48% of the domestic national product. This sector, however, is heavily dependent on timely onset, amount, duration and distribution of rainfall

(Mersha and Boken, 2005; Adhikari et al., 2015). Studies indicated that the area with stable rainfall has decreased, while the area with highly variable rainfall has substantially increased over time (Mersha, 1999; Berhan et al., 2011). The frequency and severity of this problem is likely to increase as climate change is expected to escalate drought (IPCC, 2007; Gebrehiwot et al., 2011; Adhikari et al., 2015). Lack of irrigation facilities makes the agricultural system in the country vulnerable to rainfall variability and dry spells. Dry spell probability during the main cropping season (Meher) is particularly high at the end of the season. A long-term climate data analysis revealed that altitude had a significant negative relation with temperature, but not with precipitation amount and distribution (Simane et al., 1999). Crop improvement for drought tolerance is one of the important strategies to enhance productivity and food security for the rapidly growing population.

Potato has become important crop in drought-prone highlands of Ethiopia as its short growth period makes well-suited for crop rotation with other major crops (Devaux et al., 2014; Kolech et al., 2015). Moreover, under rain fed conditions, potato yields more food per unit of water than other major crops (Monneveux et al., 2013). Consequently, it can be cultivated in environmental conditions where other crops may fail (Schafleitner et al., 2007b). These qualities make potato a strategic food security crop in Ethiopia. Despite its yield advantages over cereals, potato is more sensitive for water stress than most other crop species due to its sparse and shallow root system. Very little information is available on the actual potato yield loss due to moisture stress in Ethiopia. However, the modelling of the impact of climate change showed that potato yields are expected to decrease by 15% in Africa by 2030 (Adhikari et al., 2015). Moisture stress reduces potato growth and production by reducing the amount of productive foliage, by decreasing the rate of photosynthesis per unit of leaf area and by shortening the vegetative growth period (Van Loon, 1986; Spitters and Schapendonk, 1990).

Potato yield under water deficit conditions depends on the time, duration and severity of the stress and genotypic differences. Monneveux et al. (2013) pointed out that decline in photosynthetic rate is fast and substantial, even at relatively high water potentials (-0.3 to -0.5 MPa). Tuber initiation and bulking stage are reported to be the critical stages that are associated with the highest tuber yield loss (Vayda, 1994; Obidiegwu et al., 2015). Thus, phenotyping and selection for drought tolerance at this stage is crucially important to discriminate drought tolerant genotypes. There are genetic variations in the degree to which cultivars are affected by moisture stress. Hence, selection strategies for drought tolerant cultivars in a breeding programme are required (Mienie and De Ronde, 2008). Results from protected environment studies such as hydroponic, pot grown or greenhouse experiment may often not have direct relevance to drought tolerance in the field. Unexpected shifts in

rainfall pattern in drought prone areas, on the other hand, reduces the accuracy and effectiveness of phenotyping and phenotypic selection. Therefore, phenotyping under field conditions during the dry season could be the best solution to control over water regime and to avoid rainfall disturbance (Blum, 2011; Okogbenin et al., 2013).

Genotypes that maintain economic yields under water deficits are the target of the breeding program. Thus, desirable drought tolerant traits must be genetically associated with yield and yield components under stress. Important drought tolerance traits must also be highly heritable, genetically variable, easy to measure, stable within the measurement period, and without yield penalty under unstressed conditions (Okogbenin et al., 2013). Potato tuber yield is closely related to the plants ability to intercept solar radiation and its efficiency in dry matter accumulation. Intercepted radiation levels could be determined by leaf area (Boyd et al., 2002). Deblonde and Ledent (2000) and Schafleitner et al. (2007a) found that groundcover which is strongly related to leaf area index and biomass, was consistent with tuber yield in both drought and well-watered condition in potato. Similarly, plant height under stress could be an important growth parameter to discriminate for drought tolerance showing a good relationship with drought tolerance index (Deblonde and Ledent, 2001).

Stay green or delayed senescence in crop cultivars is recognized as important for plant production under terminal drought stress (Blum, 2011; Rolando et al., 2015). This trait can be assessed by measuring chlorophyll content. The Minolta SPAD-502 meter measures green color intensity and is a good indicator of chlorophyll concentration. It is therefore an ideal instrument for obtaining the data without destructive sampling (Uddling et al., 2007). In potato, chlorophyll content was reported to have a direct association with drought tolerance though its contribution to yield was variable (Van der Mescht et al., 1999; Blum, 2011; Yactayo et al., 2013; Ramírez et al., 2014). Similarly, canopy temperatures have been suggested as a method to identify drought tolerant potato clones as they are related to stomatal conductance and transpiration which is associated with rate of photosynthesis (Blum, 1988).

Potato is one of the most important crops in the highlands of the north-western Ethiopia. The region is known for drought occurrence covering wider proportions and affecting food security and rural livelihoods. However, there is no systematic research conducted on breeding of potato for drought tolerance and yield related traits.

Selection of potato genotypes based on combining ability estimates is useful to identify the most valuable parents and families for breeding and cultivar development. The importance of both additive and non-additive gene action in inheritance of yield and yield components have been reported in different studies under unstressed conditions (Brown and Caligari, 1989;

Maris, 1989; Ruiz de Galarreta et al., 2006). However, information is scanty under water stressed conditions. The objective of this study was therefore, to determine combining ability effects and gene action determining drought tolerance, yield and yield components among selected potato clones and to identify promising parents and crosses for cultivar development.

4.2 Materials and methods

4.2.1 Germplasm

Sixteen potato clones selected from a B3C2 population with wide genetic background and specific adaptation for highland tropics (Landeo et al., 1995) were crossed using the North Carolina Design II (NCD II) in two sets (Comstock and Robinson, 1952; Hallauer et al., 1988). Details of the clones are described in Table 4.1. Four clones were designated as female and crossed with another four clones used as male parents to form 16 families in each set. In total, 32 families were generated. Three widely grown cultivars (Belete, Guassa and Gorebella), two promising clones from B3C2 population (396038.101 and 396029.25) and 12 parental clones were also included in the study.

Table 4.1 Pedigree, root dry mass, and tuber specific gravity of potato parents used to generate families using North Carolina Design II

Clones	Set	Female/Male	Pedigree	Root dry mass(g)[a]	Tuber specific gravity
395011.2	1	Male	393085.5 x 392639.8	43.78	1.23
396041.102	1	Male	393280.58 x 393280.57	-	-
395017.229	1	Male	393085.13 x 392639.8	26.64	1.19
396038.107	1	Male	393077.54 x 393280.64	25.87	1.18
395015.6	1	Female	393083.2 x 391679.12	23.52	1.20
395109.34	1	Female	391589.26 x 393079.4	17.35	1.17
396004.263	1	Female	391002.6 x 393382.64	21.15	1.19
396034.103	1	Female	393042.5 x 393280.64	15.13	1.19
395017.14	2	Male	393085.13 x 392639.8	40.78	1.17
395077.12	2	Male	391586.109 x 393053.6	20.34	1.20
396012.288	2	Male	391004.10 x 393280.58	-	-
396264.14	2	Male	393280.82 x 392639.2	-	-
395096.2	2	Female	393085.5 x 393053.6	40.94	1.22
395109.7	2	Female	391589.26 x 393079.4	-	-
395112.32	2	Female	391686.15 x 393079.4	26.75	1.20
396031.108	2	Female	392633.64 x 393382.64	17.32	1.19

[a] Root dry mass and tuber specific gravity are estimated from previous field evaluation at Enjibara, 2013.

4.2.2 Seedling generation

A maximum of 200 F1 seeds per cross was sown in seedling trays filled with 1:2:1 mixture of sterilised sand, farmyard manure and soil, respectively in a screen house at Adet Agricultural Research Centre (11°17′ N, 37°47′ E and altitude of 2240 meter above sea level (masl)).

After 35 days, 80 to 120 seedlings of each cross were transplanted into 10 cm plastic pots for further growth. Fertilizers were applied as per recommendation and pesticides were sprayed when required.

Seedling progenies from 32 crosses having 60 genetically unique siblings each harvested separately, approximately three months after transplanting. As the tubers were harvested from each plant, they were left in the pots in which the plant was grown and one tuber from each pot was taken to produce tuber families comprised of a single tuber from each true seedling. Two sets of tuber families which were selected were saved for planting in two environments, i.e., under moisture stressed and well-watered conditions. Seed potatoes were stored at Injibara using the diffused light storage facility approximately for five months prior to field evaluation. The first moisture stress trial was established at Merawi (11°41′ N, 37°16′ E and altitude 2000 masl). However, unexpected rainfall during the dry season disrupted the experiment and evaluation was not possible. Thus the first clonal generation of F1 families were advanced to second clonal generation for the next dry season evaluation and the same procedure were followed as the seedling progenies to produce tuber families.

4.2.3 Trial establishment and experimental design

A total of 49 entries that included 32 families each having sixty progeny plus their 12 parents along with five checks were planted at Adet Agricultural Research Centre (11°17′ N, 37°47′ E and altitude of 2240 masl) during long dry season (November 4, 2014 – March 5, 2015). Trials were established using a 7x7 simple lattice design with two replications of three rows, 3 m long plots each having 30 plants. Inter- and the intra-row spacing were 0.75 and 0.3 m, respectively. The season was rain free and had a lower mean air temperatures than was experienced in the main (rainy) season of 2014. This effect, however, was offset by lower relative humidity and higher wind speed of this second season which favored evaporation (Table 4.2). Two irrigation regimes were used: under optimal soil moisture control treatment and under terminal stress. Terminal moisture stress was imposed by withholding water supply six weeks after planting until harvest. The time to impose drought was decided based on previous experience of the time of tuber initiation for most of the parents used. Iirrigation was supplied using furrows. The furrows length was limited to 3.5 m to reduce variation in water infiltration along the furrow.

The soil of the site was a clay loam with 41% of sand, 29% of clay and 30% of silt and with electronic conductivity (EC) of 0.18 mS/cm and pH of 6.35. Soil water potential was measured in depths of 0.2 and 0.4 m in both irrigated and moisture stressed plots with a granular matrix sensor (watermark sensor) (Irrometer Co., Box 2424, Riverside, CA 92516, USA). A total of 28 watermark sensors were installed for the experiment: 14 for well-watered

and 14 for water stressed experiment. Two sensors for every sub- block (row) were installed, one at the depth of 0.2 m and the other at 0.4 m. The control treatment (for the full season) and the water stressed treatment (until the 6th week) were irrigated before the soil water potential at 0.2 m depth reached 30 centibars (cb). According to Shock et al. (2013) 30 cb is the optimum soil water potential for medium textured soil at 0.2 m depth and 100 to 200 cb indicates that the soil is becoming dangerously dry and production is adversely affected. The weekly water sensor readings for irrigated and moisture stressed treatments in the drought period is presented in Figure 4.1. All the necessary agronomic practices were carried out according to the recommendations to the location. Tubers were harvested on the 5th of March (120 days after planting).

Table 4.2 Mean minimum, maximum and average temperatures, relative humidity and wind-run at Adet site during the study period.

	Mean monthly air temperature (°C)				
Months	Maximum	Minimum	Mean	Relative humidity (%)	Wind-run 100ms^{-1}
November	25.7	9.8	17.7	59.0	0.26
December	25.7	7.6	16.0	42.0	0.28
January	26.9	6.9	16.9	47.5	0.31
February	29.9	8.9	19.4	39.3	0.37
Mean	27.0	8.3	17.5	46.9	0.30

4.2.4 Data collection and analyses

Yield, yield components, growth parameters and physiological traits were measured under both irrigated and drought treatments. For growth and physiological traits, data was collected every two weeks after drought was imposed in well-watered and moisture stressed treatments. Measurements were taken from four tagged plants of parents and checks, and from all individual plants in crosses. Significant treatment x genotype interaction was observed for most of the traits at 40th day of measurement after the drought was imposed. Thus the data from this date were used to identify tolerant clones and families.

The following growth parameters were measured: plant height (PHT) measured as the distance between soil surface and the apex of the main stem in centimeter. The number of main stems (STN.) was counted in each plant. Groundcover percentage (GC) was measured using grid (0.75 m x 0.60 m) that was divided into (0.075 m x 0.06 m) squares. The quadrat was held just above the canopy and the number of squares at least half filled with green leaves were counted and then divided by the total number of squares to determine the percentage cover (Boyd et al., 2002).

Physiological measurements included: Chlorophyll content (CC) measured using SPAD-502 chlorophyll meter (Minolta Co., Ltd. Japan) on the two apical leaflets of the third fully expanded leaf of the main stem. Canopy temperature (CT) was measured by a portable infrared thermometer (Major Tech-MT694) which is designed to sense long-wave infrared radiation emitted from its target and convert it to average temperature display which can be related to transpiration. CT measurements (°C) were taken on eight clear (cloudless), windless and sunny days between 9:00 to 10:00, 10:30 to 11:30 and 6:00 to 7:00 to identify the best time for genotype discrimination. Measurements taken at 10:30 to 11:30 that show the only significant difference among genotypes are presented in this study.

Yield and yield related traits: at harvest, tubers of each plot were graded in to two categories: >30 mm (marketable), <30mm (unmarketable) and were counted and weighted. Form these, total number of tubers (per plant), marketable tuber number (per plant), total tuber yield (kg plant^{-1}), marketable tuber yield (kg plant^{-1}) and average tuber weight (g) were determined. Average tuber weight was calculated as total tuber yield divided by total tuber number.

Drought tolerance index (DTI) was determined by multiple regression of stress yield on non-stress yield and maturity score under well-watered treatments over all genotypes in the study (Bidinger et al., 1987; Blum, 2011). For every data point the deviation from the regression was calculated. High and positive deviations of the actual yield from the expected indicate relative drought resistance independent of the effect of phenology and yield potential (Blum, 2011). The relative reduction of all the measured traits was calculated as RR = (control - stressed)/control and expressed in percentage.

4.2.5 Data analyses

The data for growth, physiological and yield related traits were subjected to the general analysis of variance for all crosses, parents and checks using the GLM procedure of SAS 9.3 (SAS Institute Inc, 2011) statistical program. Two way analysis of variance was performed in a randomized complete block design (RCBD) because the relative efficiency of the lattice designs over RCBD was not significant. The Spearman correlation was used to examine the relation between the traits.

Genetic analysis was performed using the North Carolina Design II procedure (Comstock and Robinson, 1952) with SAS version 9.3 (SAS Institute Inc, 2011) for individual sets and pooled over sets to identify the significance level of general combining ability (GCA) of parents and specific combining ability (SCA) of crosses. The same linear model, explained in Chapter 3, Section 3.1.5 (Hallauer et al., 1988), was used for the analysis.

Throughout the text, variation due to males within sets, females within sets, and males x females within sets will be referred to as GCA_m, GCA_f and SCA variation, respectively. For each trait, general combining ability effect for each clone and specific combining ability effect for each F1 family were calculated according to Beil and Atkins (1967). For chlorophyll content and canopy temperatures negative estimate of combining ability (GCA and SCA) effects were taken as high in a desirable direction, while neutral or positive estimate were regarded as low or undesirable. For the rest of the traits, positive estimate of GCA and SCA effects were taken as high to identify genotypes with high yield and yield components, whereas neutral or negative estimates as low. Standard errors for GCA effects of female and male parents and SCA effects of families in each sets were calculated by using the method described by Cox and Frey (1984). The relative importance of additive (GCA) and non-additive (SCA) genetic effects in determining the performance of the progeny for each of the traits was determined by individually expressing the GCA_f mean square, GCA_m mean square, and the SCA mean square as a percentage of the treatment (crosses) mean square.

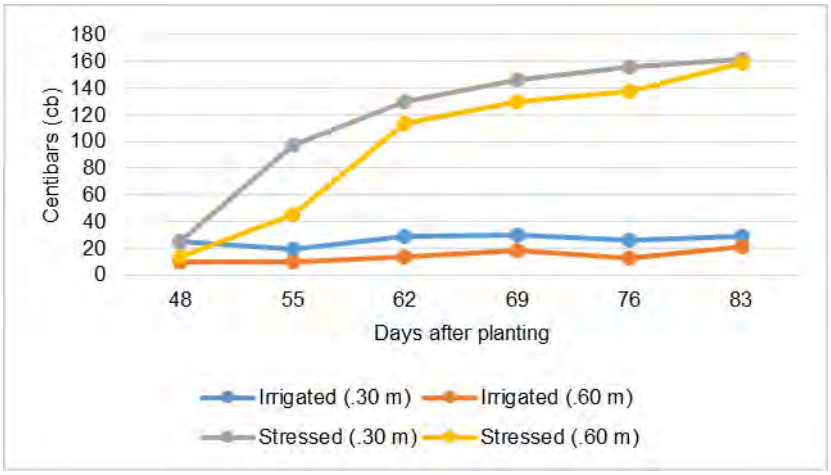

Figure 4.1 Average soil water potential measured by water mark sensor at the 0.3 m and 0.6 m soil depth for water stressed and non-stressed experiments at weekly intervals.

4.3 Results

4.3.1 Effects of water stress

Soil water potential reached 97 Centibars (cb) on the 55th day after planting which ensured the drought stress was coincided with tuber bulking (Figure 4.1). The results revealed that drought affected all the measured traits, although genotypic response differed as indicated by trait mean values and the percentage of relative reduction (RR%) for the tested population (Table 4.3). The yield reduction in the most susceptible clone 395017.14 was 57%, showing the occurrence of sufficient moisture stress that allowed discrimination of genotypes based on their drought tolerance. Drought stress had drastic effect on marketable tuber yield,

average tuber weight and total tuber yield with the corresponding relative reduction of about 51, 49 and 47%, respectively. Groundcover, number of marketable tuber per plant, and plant height were considerably less than the irrigated plants with 25, 22 and 19% reduction, respectively. Canopy temperature increased markedly under stress by 48%. Chlorophyll content and total tuber number were also increased slightly by 7, and 13%, respectively under stress condition. Number of main stems was not affected by drought as the stems were completely emerged before the stress became severe.

Table 4.3 Analysis of variance, mean values, and relative reduction (RR%) of the traits under control and drought condition

	Mean square and Significance				Means		
Traits	**Genotype (G)**	**Treatment (T)**	**G*T**	**CV (%)**	**Control**	**Drought**	**RR (%)**
TTY(kg plant^{-1})	0.031***	5.410***	0.009***	10.9	0.71	0.38	47
MTY(kg plant^{-1})	0.035***	5.816***	0.009***	11.2	0.68	0.34	51
TTN(per plant)	32.832***	47.125***	18.960***	13.2	13.62	14.6	-7
MTN (per plant)	4.633***	176.940***	1.688**	11.9	8.62	6.72	22
ATW (g)	456.550***	36022.670***	165.110***	14.3	55.16	28.05	49
PHT (Cm)	230.093***	6108.974***	30.799***	5.6	57.67	46.51	19
GC (%)	246.740***	12159.120***	59.920**	10.3	62.61	46.86	25
STN (per plant)	2.186***	0.004^{ns}	0.237^{ns}	21.6	2.95	2.96	0
CC (SPAD reading)	37.769***	1623.381***	3.432^{ns}	3.5	43.04	48.8	-13
CT(°C)	2.616***	3186.997***	4.419***	5.1	16.9	24.96	-48

***, **,*, ns = significant at P< 0.001, P< 0.01, P< 0.05 and non-significant at P> 0.05, respectively; TTY = total tuber yield; MTY = marketable tuber yield; TTN= total tuber number; MTN= marketable tuber number; ATW= average tuber weight; PHT = plant height; GC = groundcover; STN = stem number; CC = chlorophyll content; CT = canopy temperature; RR(%)= percentage of relative reduction; CV(%) = coefficient of variance; MS = mean square

4.3.2 Genetic variation under water stressed and well-watered conditions

Analysis of variance showed the existence of highly significant variation (p<0.001) among the genotypes for all the traits under both stressed and non-stressed conditions. This indicated that the populations had wide genetic background for the tested drought related traits. There were significant differences between well-watered and water stressed treatments for all the traits except for number of main stems. Highly significant interaction between the treatments and the genotypes was observed for most of the traits indicating that clones responded differently according to the water level. Chlorophyll content showed significant differences between genotypes and treatments, however, interaction between genotype and treatment was not significant.

4.3.3 Correlation of traits with tuber yield in the stressed treatments

Under drought condition, total tuber weight was significantly (P<0.001) and positively correlated with marketable tuber yield, marketable tuber number, average tuber weight, plant

height, groundcover and number of main stem (Table 4.4). High yielding clones tended to be taller, vigorous and well branched and had bigger tubers under drought condition. Canopy temperatures measured under water stressed treatment did not have strong correlation with most of the traits measured. However, the canopy temperatures measured in well-watered treatment had significant and negative correlation for total and marketable tuber yield under drought condition (r = -0.34). This indicates that genotypes with lower canopy temperatures under well-watered condition tended to have higher yield under water stressed environment. Similarly, stronger and negative correlation was obtained between total tuber weight in stressed treatment and chlorophyll content in well-watered condition (r = -0.38) than the measurement taken in treatments exposed to water stress (r = -0.28). This shows that high yielding genotypes under stress tended to lose their chlorophyll content earlier (early senescent) than the low yielding ones. Hence, canopy temperatures and chlorophyll content obtained from well-watered treatment were used for phenotyping and for combining ability estimation.

Table 4.4 Pair-wise correlation coefficients of yield and agronomic traits under water stressed condition, and physiological parameters under well-watered condition among potato genotypes evaluated at Adet, Ethiopia

	TTY	MTY	TTN	MTN	ATW	PHT	GC	STN	CC	CT
TTY	1									
MTY	0.967***	1								
TTN	-0.108^{ns}	-0.279**	1							
MTN	0.704***	0.655***	0.222*	1						
ATW	0.649***	0.772***	-0.793***	0.256*	1					
PHT	0.521***	0.492***	-0.122^{ns}	0.221*	0.398***	1				
GC	0.682***	0.676***	-0.08^{ns}	0.445***	0.471***	0.437***	1			
STN	0.329***	0.311**	0.103^{ns}	0.532***	0.121^{ns}	0.036^{ns}	0.27**	1		
CC	-0.281**	-0.313**	0.342***	-0.266**	-0.374***	0.156^{ns}	-0.169^{ns}	-0.375***	1	
CT	0.066^{ns}	0.126^{ns}	-0.312**	0.060^{ns}	0.286**	-0.145^{ns}	-0.031^{ns}	0.226*	-0.238*	1

***, **,*, ns = significant at P< 0.001, P< 0.01, P< 0.05 and non-significant at P> 0.05, respectively; TTY = total tuber yield; MTY = marketable tuber yield; TTN= total tuber number; MTN= marketable tuber number; ATW= average tuber weight; PHT = plant height; GC = groundcover; STN = stem number; CC = chlorophyll content; CT = canopy temperature

4.3.4 Drought tolerance of crosses and clones

Data for tuber yield and seven important yield determinant traits which showed high correlation to total tuber yield are presented in Table 4.5. Genotypes in Table 4.5 are sorted in descending order based on their drought tolerance index (DTI). The total tuber yield per plant ranged from 0.5 to 0.2 kg for the families of the crosses 395109.34 x 396041.102 and 395096.2 x 395017.14 respectively and from 0.6 to 0.3 kg for the clones 396038.107 and 395096.2, respectively. The clones 396038.101, 396038.107, 396029.25, Gorebella and

395112.32 were the most tolerant based on drought tolerance index. All of these clones exhibited the highest plant height and groundcover except Gorebella and 396029.25 for plant height and groundcover, in that order. Clone 396038.107 had the highest groundcover and favorable chlorophyll content (lower). Clone 395112.32 showed the highest stem number and plant height. The clones with high DTI widely differed in their canopy temperatures although all were in the lower half among the tested genotypes. The following families displayed high yield and high drought resistance index: 396034.103 x 396038.107, 395096.2 x 396012.288, 395109.34 x 396041.102, 396031.108 x 396012.288, 395109.34 x 396038.107, 395112.32 x 396012.288, 395112.32 x 396264.14, 395109.7 x 395017.14, 396034.103 x 395011.2 and 395112.32 x 395077.12.

Table 4.5 Mean responses of nine traits assessed for crosses parents and checks during 2014/2015 at Adet.

	TTY[a]	MTY	ATW	PHT	GC	STN	CC	CT	DRI
Crosses									
396034.103 x 396038.107	0.46b-f	0.42b-g	27.8i-n	37.0tu	57.4a-d	3.4a-l	44.6f-l	18.0a-f	0.11
395096.2 x 396012.288	0.48a-e	0.42b-g	26.5j-o	62.9a	58.9a-c	2.7e-q	42.9i-o	17.8a-h	0.10
395109.34 x 396041.102	0.48a-e	0.45a-d	34.5f-i	56.6a-e	51.9b-i	2.0m-q	44.5f-l	16.1i-o	0.09
396031.108 x 396012.288	0.41d-l	0.38c-j	35.3f-h	53.4c-i	48.6d-m	3.1b-n	39.7q-t	16.9d-n	0.07
395109.34 x 396038.107	0.45b-g	0.43b-f	37.0e-g	56.8a-e	49.8 c-l	2.2j-q	45.7b-h	18.3a-f	0.07
395096.2 x 395077.12	0.31m-r	0.27n-r	17.2r-t	43.8m-t	39.2m-q	2.5g-q	45.6c-i	17.6a-i	0.06
396031.108 x 395017.14	0.38f-o	0.32i-p	20.7o-r	47.7g-q	46.6e-n	3.1b-o	41.9l-r	17.5a-j	0.06
395112.32 x 396012.288	0.48a-e	0.45a-e	39.7d-f	59.9a-c	59.1a-c	3.2b-n	41.0o-s	15.8k-p	0.05
396031.108 x 395077.12	0.39e-n	0.36f-m	30.2g-k	45l-s	43.4h-q	2.5g-q	41.8m-r	16.0k-p	0.04
395112.32 x 396264.14	0.44c-h	0.39c-i	23.8k-r	50.5e-m	46.2f-n	4.2ab	43.3g-m	16.9d-n	0.04
395109.7 x 395017.14	0.43c-j	0.34f-o	22.1m-r	52.0d-l	54.9b-f	2.6f-q	43.8f-l	16.8e-o	0.04
396034.103 x 395011.2	0.40 e-m	0.33h-p	21.2n-r	43.1n-t	42.1i-q	4.2ab	42.8i-o	16.5e-o	0.02
396004.263 x 396038.107	0.31m-r	0.26o-r	18.7p-s	43.4m-t	36o-q	2.3i-q	45.7b-g	17.6a-j	0.02
395112.32 x 395077.12	0.43c-i	0.37d-l	24.9j-q	56.3a-f	51.1b-j	4.1a-c	43.4f-m	15.5n-r	0.01
396004.263 x 396041.102	0.30o-r	0.27o-r	18.1q-s	43.8m-t	37.5n-q	2.3i-q	45.5d-j	17.8a-g	0.01
395109.34 x 395017.229	0.38f-o	0.26p-r	12.6st	48.8g-p	45.9f-o	2.3i-q	41.7m-r	17.1b-l	0.00
396031.108 x 396264.14	0.31n-r	0.27o-r	25.2j-p	40.0r-t	39.5m-q	2.2k-q	43.3h-m	17.8a-g	0.00
396004.263 x 395011.2	0.34i-p	0.30 j-q	24.2k-q	42.9o-t	44.2g-p	4.1a-d	45.2e-k	15.9k-p	-0.01
395109.7 x 396264.14	0.40e-m	0.36f-m	29.3h-l	52.9c-j	54.9b-f	3.5a-j	42.9h-n	16.9e-o	-0.01
396034.103 x 396041.102	0.41d-k	0.38c-k	25.2j-p	47.6h-q	53.5b-g	4.6a	42.5j-p	15.3o-r	-0.01
395109.34 x 395011.2	0.30o-s	0.27o-r	27.9i-n	45.4k-s	40.2k-q	2.6f-q	45.2e-k	19.3ab	-0.01
395096.2 x 396264.14	0.29o-s	0.27o-r	33.7f-i	46.0j-r	36.7n-q	1.8o-q	48.0bc	17.5a-j	-0.02
395015.6 x 395017.229	0.35h-o	0.32i-q	28.3i-m	36.8tu	43.4h-q	3.4a-k	38.5s-u	17.0c-l	-0.02
395109.7 x 396012.288	0.41d-l	0.37e-l	36.1f-h	57.2a-e	50.6 b-j	4.1a-d	38.1v	17.2b-k	-0.04
395109.7 x 395077.12	0.32l-r	0.29k-r	26.7j-o	46.9h-r	39.4m-q	3.2b-m	42.1k-q	17.4b-k	-0.04
395015.6 x 396038.107	0.36h-o	0.30j-q	24.3k-q	46.4i-r	46.1f-n	2.9c-p	46.4b-e	14.7r	-0.04
395112.32 x 395017.14	0.35h-p	0.29l-r	18.2q-s	50.2e-n	48.8d-m	3.7a-g	47.2b-d	19.6a	-0.06
395096.2 x 395017.14	0.21s	0.17s	12.5st	46j-r	33.5q	1.9n-q	49.3b	18.3a-e	-0.06
396034.103 x 395017.229	0.24rs	0.16s	10.5t	36.5tu	35.7pq	2.6g-q	45.5d-i	18.5a-c	-0.06
396004.263 x 395017.229	0.25q-s	0.22rs	20.4o-r	31.2uv	44.9f-p	2.8e-p	40.7p-t	18.5a-d	-0.07
395015.6 x 395011.2	0.26p-s	0.21rs	17.3r-t	46.7i-r	36.0o-q	2.6f-q	44.1f-l	17.0c-l	-0.08
395015.6 x 396041.102	0.29o-s	0.23q-s	19.3p-s	42.1p-t	38.6n-q	3.1b-o	45.8b-f	16.5f-o	-0.09
Parents and checks									
396038.101*	0.53ab	0.51a	46.7a-c	50.1e-o	49.8c-l	2.7e-q	41.8m-r	16.2h-o	0.11
396038.107	0.55a	0.53a	51.8a	55.0b-g	67.3a	3.2b-n	38.1uv	17.0d-m	0.10
396029.25*	0.49a-d	0.46a-c	40.0c-f	54.2 b-h	41.5j-q	2.4i-q	38.9r-t	16.0j-o	0.08
Gorebella*	0.46a-f	0.42b-f	31.7g-j	37.6tu	52.3b-h	3.8a-f	41.1n-s	15.0p-r	0.02
395112.32	0.51a-c	0.48ab	44.8bd	60.8ab	53.0b-h	3.9a-e	44.5f-l	16.4g-o	0.00
Belete*	0.43c-j	0.40c-i	43.1c-e	38.3s-u	50.0c-k	2.8e-q	33.2w	15.5m-r	-0.01
Guassa*	0.36h-o	0.33h-p	23.7k-r	26.6v	38.5n-q	3.6a-i	38.3t-v	15.2o-r	-0.01
395017.229	0.39e-n	0.36f-m	28.4i-m	32.5uv	56.8 b-d	2.4h-q	41.4n-s	15.0qr	-0.01
395109.34	0.37g-o	0.36f-n	50.5ab	58.8a-d	60.5ab	1.7pq	52.6a	19.6ab	-0.01
396004.263	0.41d-k	0.39c-i	35.8f-h	41q-t	44.0g-p	3.7a-h	40.9p-s	15.8l-q	-0.03
395096.2	0.30o-s	0.26p-r	19.2p-s	46.8i-r	43.3h-q	1.5q	45.1f-k	18.4a-e	-0.03
396034.103	0.43c-h	0.41b-h	36.4e-g	49.2f-p	56.3b-e	4.5a	42.2j-p	17b-l	-0.04
396031.108	0.36g-o	0.34g-p	33.8f-i	45.5k-s	49.8c-l	3.1b-n	39.8p-t	16.1h-o	-0.06
395015.6	0.34j-p	0.29l-r	27.8i-n	52.6c-k	44.8 g-p	2.2k-q	40.8p-s	16.4f-o	-0.06
395011.2	0.34 k-q	0.28m-r	23.2l-r	31.6uv	39.5m-q	2.8d-p	40.3p-t	16.6e-o	-0.06
395077.12	0.33k-q	0.31j-q	26.2j-o	45.2l-s	40l-q	2.4h-q	42.0l-q	16.6e-o	-0.07
395017.14	0.33k-q	0.29l-r	22.0m-r	37.6tu	54.8b-f	2.1l-q	49.6b	15.7m-r	-0.09
Mean	0.38	0.34	28.1	46.5	46.9	3	43	16.9	
CV (%)	11.6	12.3	12.2	7.8	10.6	21.3	2.8	4.6	

TTY = total tuber yield; MTY = marketable tuber yield; TTN = total tuber number; MTN= marketable tuber number; ATW = average tuber weight; PHT = plant height; GC = groundcover; STN = stem number; CC = chlorophyll content; CT = canopy temperature; DTI = drought tolerance index; [a] means in a column followed by the same letter(s) are not significantly different at $P < 0.05$; * clones used as checks, the rest are parents

4.3.5 Combining ability

The analyses of variance of groundcover, plant height, marketable and total tuber yield, average tuber weight, chlorophyll content and canopy temperature following North Carolina Design II procedure pooled over sets is presented in Table 4.6. Results showed that the GCA due to females within sets (GCA_f), GCA due to males within sets (GCA_m) and SCA within sets were significant for all the traits tested. The sets also were significantly different for all the traits except for canopy temperature.

Total GCA (i.e., male plus female main effects) accounted for 83% for plant height and chlorophyll content, 68% for marketable and average tuber weight, 66% for total tuber weight, 60% for canopy and 48% for canopy temperatures. Hence, GCA effect was more important than SCA effect for all the traits except for canopy temperature. Contribution of GCA_f and GCA_m were almost the same for most of the traits assessed except for average tuber weight, marketable tuber weight and chlorophyll content where the GCA_m effects were 3, 1.6, and 1.5 times larger than GCA_f effect, respectively. However, the ratio of their mean squares (i.e., the F-test or variance ratio) were statistically non-significant.

Table 4.6 Summary mean squares and significant tests of combining ability effects for yield and growth traits under water stressed condition and for physiological traits under well-watered condition of potato genotypes evaluated at Adet, Ethiopia

Source of variation	d.f	TTW	MTW	ATW	GT	PHT	CT	CC
Set	1	0.0125*	0.018**	187.80***	144.43*	695.40***	0.093^{ns}	6.13*
Replication (set)	2	0.0003^{ns}	0.002^{ns}	104.18***	64.95^{ns}	173.14***	0.020^{ns}	13.10**
GCA_f	6	0.0160***	0.014***	77.56***	128.44***	158.50***	2.139**	20.32***
GCA_m	6	0.0147***	0.022***	235.51***	135.45***	171.05***	2.804***	30.89***
SCA	18	0.0079***	0.008***	74.39***	88.16***	32.63***	2.690***	5.32***
Error	30	0.0017	0.001	3.06	23.95	8.29	0.826	0.92
GCA/(GCA+SCA)%		65.992	68.39	67.79	59.95	83.47	47.886	82.81
Contribution of GCAf		34.340	26.43	16.79	29.18	40.15	20.719	32.86
Contribution of GCAm		31.652	41.95	50.99	30.77	43.32	27.167	49.95

***, **,*, ^{ns} = significant at P< 0.001, P< 0.01, P< 0.05 and non-significant at P> 0.05, respectively; d.f. = degree of freedom; TTW = total tuber weight; MTW = marketable tuber yield; ATW= average tuber weight; PHT = plant height; GC = groundcover; CT = canopy temperature; CC = chlorophyll content; GCA = general combining ability; GCA_f = general combining ability for female; GCA_m = general combining ability for male; SCA = specific combining ability

Analysis of individual sets showed that GCA_m effect was significantly larger than GCA_f effect only for chlorophyll content in set I (Table 4.7). Generally, the proportions of GCA variance over total treatment variance were higher in set II than in set I for all of the traits. Set II had larger mean values for total tuber yield, marketable tuber yield, average tuber weight,

groundcover, plant height and low mean value for chlorophyll content. This could indicate more drought tolerance individuals could be found in set II than set I. GCA_f effect was relatively larger than GCA_m effect for most of the traits evaluated in set I, whereas GCA_m was relatively larger than GCA_f effect in set II for all the traits except for chlorophyll content. This reflects female parents in set I and male parents in set II differ more in the traits measured than their male and female counterparts, respectively. GCA_f was non-significant for chlorophyll content in set I and canopy temperature in set II. SCA was non-significant for chlorophyll content in set II.

Table 4.7 Summary mean squares and significant tests of combining ability effects for yield and agronomic traits under water stressed condition and for physiological traits under well-watered condition of potato genotypes tested in set I and set II at Adet, Ethiopia

Source of variation	d.f.	TTY	MTY	ATW	GC	PHT	CT	CC
Set I								
Replication	1	0.0042^{ns}	0.0070^{ns}	86.81***	1.334^{ns}	100.77*	0.052^{ns}	5.23^{ns}
GCA_f	3	0.0186***	0.0154***	95.86***	103.679^{ns}	228.16***	3.010**	0.71^{ns}
GCA_m	3	0.0135**	0.0218***	114.11***	70.696^{ns}	129.11***	2.500*	23.15***
SCA	9	0.0084**	0.0110***	102.39***	88.735^{ns}	36.26*	3.497***	8.15**
Error	15	0.0017	0.0017	2.15	35.778	12.13	0.549	1.53
CV (%)		11.778	13.5466	6.39	13.612	7.9	4.324	2.81
Mean		0.3495	0.3006	22.95	43.941	44.07	17.137	44.01
R^2		0.8739	0.8972	0.98	0.711	0.89	0.854	0.87
GCA/(GCA+SCA)%		65.5515	62.8355	50.63	49.56	83.12	44.066	59.4
GCAf/GCAm ratio		1.3768	0.7059	0.84	1.467	1.77	1.204	0.03**
Set II								
Replication	1	0.0018^{ns}	0.0006^{ns}	26.19*	104.904**	73.44**	0.184^{ns}	8.02*
GCA_f	3	0.0133**	0.0118***	59.27***	153.191***	88.84***	1.268^{ns}	39.93***
GCA_m	3	0.0159***	0.0214***	356.91***	200.199***	212.98***	3.109**	38.62***
SCA	9	0.0074**	0.0053**	46.39***	87.584***	29.00**	1.883*	2.48^{ns}
Error	15	0.0015	0.001	3.6	10.958	4.92	0.533	1.03
CV (%)		10.292	9.646	7.19	7.051	4.38	4.242	2.34
Mean		0.3775	0.3339	26.38	46.946	50.66	17.213	43.39
R^2		0.8732	0.9047	0.97	0.922	0.94	0.791	0.95
GCA/(GCA+SCA)%		66.4819	75.8768	81.77	66.859	83.88	53.75	94.06
GCAf/GCAm ratio		0.8377	0.553	0.17	0.765	0.42	0.408	1.03

**, **,*, ^{ns} = significant at P< 0.001, P< 0.01, P< 0.05 and non-significant at P> 0.05, respectively; d.f. = degree of freedom; TTW = total tuber weight; MTW = marketable tuber yield; ATW= average tuber weight; PHT = plant height; GC = groundcover; CT = canopy temperature; CC = chlorophyll content; GCA = general combining ability; GCA_f = general combining ability for female; GCA_m = general combining ability for male; SCA = specific combining ability; R2= coefficient of determination

4.3.6 General combining ability effects of parents

Estimates of the GCA effects for the 16 parents are shown in Table 4.8. The GCA estimates for total tuber weight per plant ranged from 0.052 for clone 395109.34 to -0.048 for clone 396004.263 in Set I and from 0.065 for clone 396012.288 to -0.052 for clone 395096.2 in set II. The parents which possessed good GCA effects for yield under drought stress were the clones 395109.34, 396034.103 and 395112.32 among female parents and clone 396012.288 and 396038.107 among male parents, which had significant and positive estimates. These parents also showed positive GCA estimate for marketable tuber yield, average tuber weight, groundcover and plant height. Male parent 396012.288, which had the highest positive GCA effect and female parent 396034.103 showed negative estimate for canopy temperature and chlorophyll content. Parent 395109.34 had undesirable and positive GCA effect for canopy temperature whereas parent 396038.107 had positive and undesirable GCA effect for chlorophyll content. Parents 396004.263, 395096.2 and 395017.14 displayed undesirable GCA effects for all the traits measured. Parents 395015.6 and 395017.229 also had undesirable GCA effects for most of the traits although they had significant desirable effect for canopy temperature and chlorophyll content, respectively.

Table 4.8 Estimates of general combining ability (GCA) effects of 32 potato parents in two sets for seven traits assessed at Adet, Ethiopia.

Parents	TTY	MTY	ATW	GC	PHT	CT	CC
			Set I				
Female							
395015.6	-0.034**	-0.035**	-0.657	-2.932	-1.056	-0.837**	-0.314
395109.34	0.052**	0.052**	5.053**	3.019	7.827**	0.571*	0.247
396004.263	-0.048**	-0.038**	-2.601**	-3.297	-3.747**	0.315	0.261
396034.103	0.029*	0.02	-1.795**	3.21	-3.024**	-0.049	-0.195
Male							
395011.2	-0.024	-0.021	-0.29	-3.319	0.461	0.052	0.286
395017.229	-0.044**	-0.063**	-5.015**	-1.465	-5.740**	0.645**	-2.413**
396038.107	0.045**	0.052**	3.974**	3.368	1.829	0.022	1.570**
396041.102	0.023	0.032**	1.330**	1.415	3.451**	-0.720**	0.557
SE	0.013	0.012	0.449	1.831	1.066	0.227	0.379
Set II							
Female							
395096.2	-0.052**	-0.050**	-3.914**	-4.884**	-1.008	0.592	3.041**
395112.32	0.046**	0.043**	0.282	4.323**	3.550**	-0.271	0.314
395109.7	0.012	0.008	2.154**	2.995**	1.598*	-0.171	-1.656**
396031.108	-0.005	-0.001	1.478*	-2.435*	-4.140**	-0.15	-1.699**
Male							
395017.14	-0.036**	-0.054**	-8.020**	-1.013	-1.701*	0.840**	2.150**
395077.12	-0.012	-0.009	-1.629**	-3.670**	-2.657**	-0.608**	-0.146
396012.288	0.065**	0.070**	8.012**	7.322**	7.675**	-0.292	-2.981**
396264.14	-0.017	-0.008	1.637**	-2.639**	-3.317**	0.06	0.977**
SE	0.012	0.01	0.581	1.014	0.679	0.224	0.31

SE = standard error; *, ** significantly different from zero at ≥ 1.96SE and 2.56SE respectively; TTY = total tuber yield; MTY = marketable tuber yield; ATW= average tuber weight; GC = groundcover; PHT = plant height; CT = canopy temperature; CC = chlorophyll content

4.3.7 Specific combining ability effects and mean response of families

Families from the following crosses: 395109.34 x 396041.102, 395015.6 x 395017.229, 396004.263 x 395011.2 in set I and 395096.2 x 396012.288, 395109.7 x 395017.14 and 396031.108 x 395017.14 in set II showed significant and desirable SCA effect for most of the traits measured (Table 4.9). The clone from cross 396034.103 x 395011.2 also had significant and desirable SCA effect for total tuber weight. All of these families had desirable SCA effect for chlorophyll content and/or canopy temperature. Among these families 395109.34 x 396041.102, 395096.2 x 396012.288, 395109.7 x 395017.14 and 396031.108 x 395017.14 were drought tolerant with the highest DTI value.

Table 4.9 Estimates of specific combining ability effects (SCA) of 32 F1 potato families evaluated in two sets for yield, yield components and drought related traits at Adet, Ethiopia.

Crosses	TTY	MTY	ATW	GC	PHT	CT	CC
Set I							
395015.6 x 395011.2	-0.029	-0.036	-4.722**	-1.69	3.255*	0.656	0.095
395015.6 x 395017.229	0.082**	0.114**	11.028**	3.844	-0.464**	0.073*	-2.752**
395015.6 x 396038.107	-0.003	-0.014	-1.932**	1.722	1.549	-1.596**	1.13
395015.6 x 396041.102	-0.049**	-0.063**	-4.374**	-3.875	-4.341**	0.867*	1.528**
395109.34 x 395011.2	-0.080**	-0.058**	0.211	-3.407	-6.929**	1.584**	0.617
395109.34 x 395017.229	0.02	-0.032	-10.388**	0.428	2.609	-1.236**	-0.198
395109.34 x 396038.107	0.003	0.02	4.978**	-0.551	3.077	0.563	-0.142
395109.34 x 396041.102	0.057**	0.069**	5.200**	3.53	1.243	-0.911**	-0.276
396004.263 x 395011.2	0.063**	0.062**	4.150**	6.83	2.124	-1.604**	0.634
396004.263 x 395017.229	-0.008	0.019	5.055**	5.748	-3.348**	0.397	-1.137*
396004.263 x 396038.107	-0.034	-0.051**	-5.668**	-8.018	1.244	0.124	-0.146
396004.263 x 396041.102	-0.021	-0.029	-3.537**	-4.56	-0.02	1.083**	0.65
396034.103 x 395011.2	0.046*	0.032	0.361	-1.732	1.55	-0.637	-1.345*
396034.103 x 395017.229	-0.093**	-0.101**	-5.695**	-10.02	1.202	0.767*	4.088**
396034.103 x 396038.107	0.035	0.045*	2.623**	6.847	-5.871**	0.909**	-0.841
396034.103 x 396041.102	0.013	0.023	2.711**	4.905	3.118	-1.039**	-1.901**
SE	0.019	0.019	0.686	2.798	1.629	0.347	0.579
Set II							
395096.2 x 395017.14	-0.076**	-0.062**	-1.961*	-7.567**	-1.987	-0.33	0.671
395096.2 x 395077.12	0.001	0	-3.626**	0.822	-3.232**	0.409	-0.643
395096.2 x 396012.288	0.089**	0.065**	-4.007**	9.505**	5.589**	0.281	-0.579
395096.2 x 396264.14	-0.014	-0.003	9.594**	-2.76	-0.37	-0.36	0.551
395112.32 x 395017.14	-0.037*	-0.034*	-0.46	-1.465	-2.305*	1.824**	1.298
395112.32 x 395077.12	0.018	0.004	-0.087	3.456*	4.754**	-0.862*	-0.174
395112.32x 396012.288	-0.011	0.005	5.030**	0.464	-2.023	-0.855*	0.254
395112.32 x 396264.14	0.03	0.025	-4.483**	-2.455	-0.426	-0.108	-1.378
395109.7 x 395017.14	0.072**	0.056**	1.534	5.981**	1.409	-1.081**	-0.05
395109.7 x 395077.12	-0.053**	-0.042**	-0.225	-6.882**	-2.655*	0.919**	0.536
395109.7 x 396012.288	-0.047**	-0.044**	-0.449	-6.686**	-2.721**	0.411	-0.687
395109.7 x 396264.14	0.028	0.029	-0.86	7.587**	3.968**	-0.25	0.202
396031.108 x 395017.14	0.040*	0.039**	0.887	3.051*	2.883**	-0.413	-1.919
396031.108 x 395077.12	0.034	0.038*	3.938**	2.603	1.134	-0.467	0.281
396031.108 x 396012.288	-0.03	-0.026	-0.573	-3.283*	-0.845	0.162	1.013
396031.108 x 396264.14	-0.044*	-0.051**	-4.251**	-2.372	-3.172**	0.718*	0.625
SE	0.018	0.015	0.887	1.548	1.038	0.342	0.474

SE = standard error; *, ** significantly different from zero at ≥ 1.96SE and 2.56SE respectively; TTY = total tuber yield; MTY = marketable tuber yield; ATW= average tuber weight; GC = groundcover; PHT = plant height; CT = canopy temperature; CC = chlorophyll content

4.4 Discussion

4.4.1 Treatment effects

Drought reduced yield of all potato genotypes in the present study. Genotypes had reduced marketable tuber number and yield, tuber size, plant height and groundcover due to drought. Contrary to previous reports (Lahlou et al., 2003; Schafleitner et al., 2007a; Cabello et al., 2014), total tuber number increased by 7% under drought stress. Haverkort et al. (1990) found that, in both controlled and field conditions, drought before tuber initiation increased total tuber number, while the number of tubers remained unchanged when drought occurred during tuber initiation. Yield under drought stress had not shown any relation to total number of tubers in the present study, indicating that yield reduction due to the stress was associated

instead, with distribution of tuber size. Drought caused undesirable downward shift in tuber size distribution, which reduced the number of marketable tubers (>30mm). This was confirmed by stronger and negative correlation of total tuber number with average tuber weight ($r = -0.79$, $P<0.001$) as compared to their correlation under well-watered condition ($r= -0.65$, $P<0.001$). A similar result was reported by MacKerron and Jefferies (1988). Cabello et al. (2014) also indicated that total tuber number was a poor predictor of tuber yield under stress.

Chlorophyll content, which is the indicator of delayed senescence, increased significantly (13%) in response to the stress. Similar results were observed by Rolando et al. (2015) and Ramírez et al. (2014) in potatoes. The increase in chlorophyll content could be explained by turgor loss or a reduction of leaf growth (Teixeira and Pereira, 2007; Rolando et al., 2015). Reduction of leaf growth was confirmed by 25% groundcover decrease under stress (Table 4.2). However, the interaction of genotypes and moisture level was non-significant, showing genotypes had increased their chlorophyll content under stress in a similar manner. Blum (2011) and Rolando et al. (2015) pointed out that stay green (non-senescence) is largely a constitutive trait which can be expressed under well-watered conditions, and is highly heritable which makes it an easily manipulated trait for breeding. Canopy temperature increased due to stress. Decreased water uptake due to soil water depletion closes stomataes, which reduces transpiration and increases leaf temperature (Blonquist Jr. et al., 2009). Leaf-canopy temperature is a reliable indicator of plant water stress (Blum et al., 1982; Blum, 2011)

4.4.2 Relation between yield and other traits

High yielding genotypes were better able to maintain their marketable tuber yield, marketable tuber number, tuber size, plant height, and groundcover under water stress. Among the secondary traits tested, groundcover seems to be the major determinant of yield under stress followed by plant height ($r = 0.68$, $P<0.001$ and $r = 0.52$, $P<0.001$, respectively). A similar result has been reported by Schafleitner et al. (2007a) using vegetative indices which are related to leaf area index and above ground biomass. Rolando et al. (2015) and Boyd et al. (2002) concluded that genotypes which show less reduction in growth and carbon assimilation rate could also show less tuber yield reduction under stress. The measurement of groundcover has the advantage of being quick and non-destructive.

In the present study, loss of chlorophyll content and cooler canopy in irrigated treatment had significant relationship with high yield under stress. Contrasting results have been reported in contribution of chlorophyll content (stay green trait) to yield under stress (Anithakumari et al., 2012). Loss of chlorophyll content related to resource remobilization in pea and soybean

(Blum, 2011). Ramírez et al. (2014) found that a slower rate of chlorophyll concentration reduction and increased leaf greenness at early senescence were negatively correlated with tuber yield under water restriction in potato. Blum (2011) explained that resource mobilization capacity to the harvested organs of the plant, which leads to higher yield, is mutually exclusive with stay-green.

Likewise, the correlation between low canopy temperature in irrigated treatment and high yield in stressed condition suggests that increased stomatal conductance is a desirable trait to improve yield under both stressed and non-stressed environment. Low canopy temperature related to high stomatal conductance and transpiration, which is associated with increased rate of photosynthesis. Early stomatal closure in response to drought causes a similar reduction in growth rate and final yield (Blum, 1988; Rolando et al., 2015). Reynolds et al. (1994) found that higher yielding wheat genotypes under different soil moisture condition showed lower canopy temperature under well-watered environment. Selection for low-transpiration types may translate to selection for low yield depression under stress, but would result in lower yields under optimum conditions (Spitters and Schapendonk, 1990). The present study demonstrated that the yield improvement in water-limited environment would not necessarily be associated with traits that cause yield penalty in high yielding environment.

4.4.3 Variation in drought tolerance among the tested genotypes

In the present study, maturity assessed on the 90th days after planting had significant (r = -0.40) correlation with tuber yield showing that early maturing genotypes had better yield advantage over the lately matured ones. Highly significant (r = 0.60) correlation was also observed between yield under well-watered and yield under stress. Given the strong effects of phenology and yield potential on yield under the stress treatment in this study, it is clear that actual tuber yield under stress *per se* is of little value in describing a genotype's drought tolerance. Regression analysis of the combined effects of phenology and yield potential on tuber yields in the stress treatments indicated that these two factors accounted for 36% of the observed variation in tuber yield under the stress (Data not shown). Therefore, in this study drought tolerance index (DTI) which is regressed for yield under stress and maturity was used to identify drought tolerant genotypes and crosses. This parameter could assure the yield achieved under stress was due to drought tolerance, instead of drought escape and is independent on yield potential.

DTI was positively correlated to yield in the drought treatment. Conversely, the index is unrelated to yield under well-watered treatment, indicating that breeding for stress tolerance would not necessarily have negative effects on yield under non-stressed conditions for the

population studied. Based on the DTI value and yield under stress the following clones were classified as drought tolerant and high yielding under stress: 396038.101, 396038.107, 396029.25, Gorebella and 395112.32. Among these, 395112.32, 396038.107 and commercial cultivar Gorebella were the best yielders both under drought and well-watered condition. The genotypes 396029.25 and 396038.107 had the additional merit of being late blight resistant and a good combiner for later blight resistance, respectively (Chapters 3 and 4). Consequently, clone 396029.25 could be recommended for release in drought prone areas during the rainy season. Clone 396038.107 could also be a good parent to generate progenies with combined drought tolerance and late blight resistance. The study showed that the three local cultivars: Gorebella, Belete and Guassa had moderate to high levels of drought tolerance. This could be associated with the presence of drought related traits in these genotypes, since they were selected for yield stability across broad range of environments with variability in rainfall amount and distribution (Woldegiorgis, 2013; Kolech et al., 2015).

4.4.4 Gene action and combining ability

The study showed highly significant GCA and SCA effects for total tuber yield, marketable tuber yield, average tuber weight, plant height, groundcover and canopy temperature, indicating the importance of both additive and non-additive gene action in conditioning these traits. Larger GCA than SCA effects for total tuber yield (60%), marketable tuber yield (68%), average tuber weight (68%), plant height (83%), chlorophyll content (83%) and groundcover (60%), indicated that additive genetic effect predominantly control the phenotypic variation of these traits under moisture stress. There have been few studies on the inheritance of potato yield, yield components and drought related traits under moisture stress. Recently Cabello et al. (2014) reported the predominance of additive genetic variance over non-additive genetic for average tuber weight. In wheat, additive gene effects have been demonstrated in the genetic control of flag leaf area duration which is corresponded to ‘stay green’ effect (Simon, 1999). Anithakumari et al. (2012) were able to identify three quantitative trait loci (QTL) for chlorophyll content. These traits can be effectively improved by appropriate selection procedures. For canopy temperature non-additive effect was predominant over additive effect (GCA effect = 48%), thus specific hybridization should be considered to enhance this physiological trait.

The study showed that the following clones: 396038.107, 396034.103, 396012.288, 395109.34, and 395112.32 contributed to high GCA effect for yield and most desirable traits for drought tolerance in their respective sets and were parents to the most resistant families as measured by DTI. Among these clones: 395112.32, 396034.103 and 396012.288 had negative GCA effect for chlorophyll content and/or canopy temperature, indicating that they

can be utilized as breeding parents for stress tolerance that does not compromise yield. The yield potential of clones 395112.32 and 396034.103 was confirmed by their high *per se* performance under well-watered experiment. On the other hand, clone 395109.34, which showed undesirable GCA canopy temperature and chlorophyll content, was among the lowest yielding genotypes under well-watered treatment, suggesting that it could be associated with undesired "static" yield stability which cause yield penalty if high rainfall occurs. Schafleitner et al. (2007a) explained that cultivars with minimal yield losses under drought might have a low yield potential if their resistance is associated with stay green and/or high canopy temperature. Nevertheless, these cultivars might harbour interesting drought tolerance traits that could be transferred to higher yielding commercial varieties. In addition clones 395017.229, 395109.7 and 396031.108, which had significant and negative GCA effect for chlorophyll content, can also be utilized for breeding for high yields, because loss of chlorophyll content was found to be correlated with yield both under stress and well-watered condition. Clone 396041.102 with high and significant GCA effect for marketable tuber yield, average tuber weight, plant height and canopy temperature could be an important donor for yield components and cooler canopy temperature.

Families from crosses of 395109.34 x 396041.102, 395096.2 x 396012.288, 395109.7 x 395017.14, 396031.108 x 395017.14 were among the most drought tolerant crosses which showed significant SCA effect for most yield and drought related traits. Most of the crosses with high yield and drought tolerance were obtained from the parental combination with different desirable characters. For instance, the family 395109.34 x 396041.102 had high GCA effect of female parent 395109.34 for yield and growth traits in moisture stressed condition complementing with the desirable GCA effect of the male parent 396041.102 for canopy temperature. The high SCA effect observed in crosses involving 395096.2 and 395017.14 could be explained by their complementary effects for high root dry mass (Table 4.1) with the desired GCA effect of their male and female counterparts for yield components and physiological traits, respectively. Larger roots and deeper roots provide better access to remaining soil water. Different combinations of drought tolerance traits may lead to the same effect, which is tuber yield maintenance under drought condition (Schafleitner et al., 2007b). Effective crop improvement for drought tolerance will require the pyramiding of many complementary characters, with different combinations (Obidiegwu et al., 2015). Among the best yielders and drought tolerant families, 395096.2 x 396012.288 showed good SCA effect for late blight resistance (Chapter 4), suggesting that the progenies from this combination would be high yielding, drought tolerant and late blight resistant.

4.5 Conclusions

The present study showed that the parents 396034.103, 396012.288 and 395112.32 were good combiners for tuber yield under stress and most drought related traits that do not affect yield under non-stressed situation. Families from crosses of 395109.34 x 396041.102, 395096.2 x 396012.288, 395109.7 x 395017.14, 396031.108 x 395017.14 were selected for their best SCA effects for high yield and drought tolerance. The selected parents and families were the best candidates to develop improved potato varieties for drought prone areas of the north-western Ethiopia or similar environments. The predominance of variance due to GCA over SCA effects suggests that high response to selection would be obtained either by directly selecting for yield or for desirable traits correlated with yield. Overall, results indicated that it would be possible to breed improved potato cultivars with combined drought tolerance and high yield potential.

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CHAPTER 5. Genetic diversity analysis of selected potato genotypes using SSR markers

Abstract

Knowledge about diversity of genetic resources is important for an efficient choice of parents. The objective of this study were to assess the level of genetic diversity among eighteen selected potato clones using 23 simple sequence repeat (SSR) markers and to complement phenotypic selection for identification of suitable parents for breeding. The results showed that pair-wise estimates of similarity ranged from 0.26 to 0.52 with the mean of 0.35. Ninety-five alleles were amplified by twenty three SSR primer pairs among all genotypes. Polymorphic alleles per locus ranged from 3 to 7 with a mean of 5. The polymorphic information content (PIC) values of loci ranged from 0.15 to 0.81 with a mean of 0.62. Observed heterozygosity (Ho) varied from 0.17 to 1 with an average of 0.78. Mean unbiased expected heterozygosity was 0.68. Cluster analysis and principal coordinate analysis separated the genotypes into three distinct groups. The following clones: 396029.25 from cluster I, clone 396038.107, 396038.101 and 395112.32 from cluster II, and clones 395017.229 and 395109.34 from cluster III, could be promising parents for breeding with high to moderate late blight resistance and drought tolerance. The present study showed that the tested potato genotypes had wide genetic diversity and hybridization between the highly differentiated clones could render superior genetic combination.

Keywords: genetic diversity, polymorphic information content, simple sequence repeat (SSR) markers, tetraploid potatoes.

5.1 Introduction

Potato is the world's number one non-cereal food crop with production reaching 376 million tons in 2013 (FAOSTAT, 2015). Because of its high yield and food value per unit area as compared to most cereals and its short growth period, potato is considered as a potential food security crop for the growing population of the world (Hoque et al., 2014). Ethiopia is among the top ten potato producer countries in sub-Saharan Africa and the production is increasing over time. However, the average national yield is 11 t ha^{-1} which is far below the attainable yield of over 40 t ha^{-1} (Woldegiorgis, 2013). Drought and late blight disease are the major impediments contributing to extensive crop loss in Ethiopia. Given that potato is mostly produced by small scale farmers who cannot afford chemical control and irrigation, genetic improvement is the best option to increase productivity of potatoes.

The cultivated potato, *Solanum tuberosum* subsp. *tuberosum*, is a highly hererozygous autotetraploid species (2n = 4x = 48), with a genome size of 844 Mb, is a predominantly outcrossing species and suffers acute inbreeding depression (Stupar et al., 2007; Park et al., 2009). The crop shows tetrasomic inheritance which increases the number of progeny genotype classes and allelic dosage (Luo et al., 2000). Potato cultivars are obtained from crossing heterozygous parents and selecting among the F1 progeny. Heterozygous genotypes are immediately fixed due to vegetative propagation via tubers (Bradeen and Kole, 2011). Thus, a promising clone can be multiplied with all its favourable inter- and intra-allelic gene actions intact. Potato exhibits heterosis due to multi-allelic gene action. Heterozygosity in potato is known to be essential to realize heterosis for economic traits like tuber yield (Gopal and Minocha, 1997). Gopal (2015) pointed out that the inbreeding coefficient is negatively associated with vigour and tuber yield.

An understanding of the breeding material allows breeders to select the appropriate parents to be used in designed crosses (Acquaah, 2007). Potato breeders have used a number of approaches for the selection of superior parents and cross-combinations. These include use of mid-parent values, combining ability effects, estimated breeding values, progeny tests and genetic diversity (Gopal, 2015). Owing to the complexities in genetics and inheritance pattern of potato, various strategies for the selection of parents need to be used in combination to attain reliable results (Sharma and Nandineni, 2014; Gopal, 2015).

High level of genetic diversity among potato genotypes possessing different desirable traits would greatly benefit breeding programmes for further improvement. Selection of parents based on genetic diversity is a good strategy to maximize heterozygosity, to broaden the genetic base and to produce heterotic progenies (Gopal and Minocha, 1997). Conversely, a narrow genetic base cause loss of fitness or inbreeding depression as a consequence of accumulation of deleterious alleles in a population (Gopal, 2014). The assessment of genetic diversity can be achieved through pedigree, phenotypic, biochemical and/or molecular information (Govindaraj et al., 2015). Molecular marker systems are stable, and not affected by the developmental stage of the plant. They are also independent to environmental, pleiotropic, and epistatic effects (Govindaraj et al., 2015). A number of molecular markers including random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphism (RFLPs), amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs) have been developed and used to complement phenotyping (Mondini et al., 2009).

Microsatellites or simple sequence repeats (SSR) DNA markers are currently the robust molecular approach to study genetic diversity. They are relatively abundant, co-dominant,

multi-allelic, and highly polymorphic, even among closely related cultivars due to mutations causing variations in the number of repeating units (Spooner et al., 2005). Microsatellites provide high genetic information, are highly reproducible, and simple to use. Additionally, the SSRs have the capacity to reflect ploidy status and the level of heterozygosity of the tetraploid potatoes (Muthoni et al., 2014).

Diversity assessment using molecular markers complement phenotypic information for parental selection to ensure genetic variation for continued progress (Acquaah, 2007). Hence the present study was undertaken to assess the genetic diversity among eighteen potato clones that show variability for drought and late blight resistance using SSR markers. This study will help to complement phenotypic data with molecular information that is neutral in terms of environmental influences and conducive to quantitative estimates of genetic similarity and distance.

5.2 Materials and methods

5.2.1 Plant materials

Eighteen potato clones, belonging to population B group 3, cycle two (B3C2), which have been developed by the International Potato Centre (CIP) for durable late blight resistance, were used in the study. Among which one clone 393371.58 (Belete) was tested under Ethiopian condition and release as a cultivar in 2010, while the rest of the17 genotypes are advanced clones. The clones have different reaction for drought and late blight. A list of potato clones used in this study and their description is presented in Table 5.1.

Table 5.1 A list of 18 potato clones used in the study, their late blight reaction and drought tolerance level

No.	Clones	Late blight reaction	Drought tolerance
1	395011.2	Moderately resistant	Sensitive
2	395015.6	Susceptible	Sensitive
3	395017.14	Susceptible	Sensitive
4	395017.229	Moderately resistant	Moderately tolerant
5	395077.12	Moderately resistant	Sensitive
6	395096.2	Moderately resistant	Sensitive
7	395109.34	Moderately resistant	Moderately tolerant
8	395112.32	Susceptible	Tolerant
9	396004.263	Resistant	Sensitive
10	396034.103	Moderately resistant	Sensitive
11	396038.107	Susceptible	Tolerant
12	396031.108	Moderately resistant	Sensitive
13	392633.64	Susceptible	-
14	393220.54	Resistant	-
15	396029.25	Resistant	Tolerant
16	396038.101	Susceptible	Tolerant
17	396038.105	Resistant	-
18	393371.58 (Belete)	Resistant (Unstable)	Moderately tolerant

5.2.2 Genotyping

For the molecular diversity assessment, a set of 23 polymorphic microsatellite markers were used. The details of the markers are shown in Table 5.2. These markers were obtained from the recently selected 24 potato genetic identity (PGI) kit set up by Ghislain et al. (2009). This kit has been proposed for use as a reference for standardizing the potato germplasm analysis across laboratories. The markers were selected based on quality of amplicons as determined by clarity and reproducibility, genome coverage, and locus-specific polymorphic information content. The kit provides two markers from each of the 12 linkage groups of potato separated by at least 10 cM (Ghislain et al., 2009).

Genomic DNA was obtained from the DNA bank of CIP. The loci were amplified using standard protocol for SSR markers from CIP (Ghislain et al., 2004). Genotyping was conducted using a LI-COR 4300 DNA Analysis System and SSR allele scoring was performed using SAGA Generation 2 software (LI-COR) (Ghislain et al., 2009).

5.2.3 Data analysis

Genotypic data were subjected to various analysis of the genetic diversity of clones using GENALEX version 6.5 (Peakall and Smouse, 2007). The chi-square (x^2) test was performed to determine the differences in allele frequencies among the SSR markers. Genetic diversity parameters, such as the total number of alleles per locus (N_a), the number of effective alleles per locus (N_e), allelic richness (Ar), observed heterozygosity (H_o), expected heterozygosity (H_e), were determined using the protocol of Nei and Li (1979). Allelic richness was corrected

for sample size differences and estimated by using the rarefaction method implemented in HP-Rare 1.0 (Kalinowski, 2005). The polymorphic information content (PIC), is a measure of allelic diversity and was calculated as PIC=1-$\sum pi^2$, where *pi* is the frequency of i^{th} allele detected in all individuals of the populations (Nei, 1973).

Two approach were adopted to investigate the genetic structure and diversity among the potato clones. In the first approach, polymorphisms were treated as binary data. The SSR marker alleles were scored as discrete variables, 1 for presence or 0 for absence of the band for all the 18 potato genotypes. Each SSR band amplified by a given primer was treated as a locus. The binary data were used to obtain a dissimilarity matrix using the Jaccard index. The matrix was used to run a cluster analysis based on Neighbor-Joining employing the software DARwin 5.0 (Perrier and Jacquemoud-Collet, 2006). A dendrogram was generated on the dissimilarity matrix. The binary data were also used to generate the principal coordinate analysis (PCoA). The plot was generated from the first two principal coordinates highlighting the distance between the different potato varieties based on scores. The reliability of the dendrogram was tested by bootstrap analyses with 10,000 replications to assess branch support. The second approach based on the co-dominant nature of the marker was used to determine the genetic structure within and among accessions using GENALEX version 6.5.

5.3 Results

The statistics of genetic diversity parameters are given in Table 5.2. The 18 potato genotypes evaluated in this study were differentiated uniquely, using the 23 SSR markers. Each of the markers differed significantly in their ability to determine variability among the clones. Some markers generated several alleles, while others generated only a few. A total of 95 putative alleles were detected across 18 potato clones using the 23 SSR markers. The maximum number of polymorphic alleles (7 alleles) was obtained from 22% of the markers, while the minimum number of polymorphic alleles (3 alleles) was amplified from 17% of the markers. The mean number of polymorphic alleles per marker was 5. The overall size of the amplified product varied from 91 bp (marker STM0037) to 314 bp (STM5114). The size difference between the smallest and the largest allele at a given SSR locus varied from 4 (STM1053) to 44 bp (STM1106). Null alleles were observed only from few markers. Among the genotypes assessed in the panel, the highest frequency of null alleles was observed from CIP395096.2. In this genotype 8 (35%) of the markers failed to produce detectable amplification. Significant variation was observed among effective alleles. Genetic diversity can be measured by the effective number of alleles, which is the number of alleles that would be maintained if all alleles had the same frequency. The number of effective allele (Ne) ranged from 1.2 (STM1064) to 5.5 (STM0037) with a mean of 3.5 alleles per locus.

The level of polymorphism among the 18 potato clones was evaluated by calculating PIC values for each of the 23 SSR loci. The PIC values significantly varied among loci and ranged from 0.15 for primer STM1053 to 0.81 for STM0037, with a mean value of 0.62 per locus (Table 5.2). PIC values showed a significant positive linear correlation with number of alleles at SSR locus ($r = 0.77$; $p < 0.001$). Over 83% of the SSR-loci had PIC value of >0.5 and about 57% of the loci had PIC values of >0.7, indicating an adequate discriminatory power of individual SSR loci used in the study.

The results of the x^2 test showed significant differences in allele frequencies at all loci for all the genotypes. The probability that two randomly selected alleles in a given genotype are different, estimated by H_e, was found to be 0.68, with maximum and minimum values of 0.16 and 0.84 recorded by the microsatellite markers STM1064 and STM0037, respectively. The observed heterozygosity value (H_o) at each locus ranged from 0.17 (STM1064 and STPoAc58) to 1.00 (STM5114, STI0033, STI0014, STG0016, STG0010, STI0003 and STM0037), with a mean value of 0.78 for all the loci.

Table 5.2 Chromosome location, allele size range and genetic diversity parameters for 23 simple sequence repeat loci used in the study

Marker	Motif	Chromosome	Allele size range (bp)	Genetic parameters						
				Na	Ne	Ho	He	F	PIC	Ar
STG0001	$(CT)_{10}$	XI	145-158	7	4.31	0.88	0.79	-0.15	0.75	5.56
STG0010	$(TG)_6$	III	178-187	5	4.07	1	0.78	-0.33	0.74	4.52
STG0016	$(AGA)_8$	I	143-175	6	4	1	0.77	-0.33	0.73	4.52
STG0025	$(AAAC)_5$	X	216-221	3	2.44	0.88	0.61	-0.5	0.51	3.33
STI0001	$(AAT)_n$	IV	196-210	5	4.32	0.94	0.79	-0.23	0.75	4.74
STI0003	$(ACC)_n$	VIII	140-177	6	4.44	1	0.8	-0.29	0.75	5.77
STI0004	$(AAG)_n$	VI	96-122	5	3.75	0.78	0.75	-0.06	0.7	4.53
STI0012	$(ATT)_n$	IV	184-208	7	5.02	0.94	0.82	-0.18	0.79	5.38
STI0014	$(TGG)n(AGG)_n$	IX	140-149	4	3.83	1	0.76	-0.35	0.72	3.7
STI0030	$(ATT)_n$	XII	106-126	7	4.53	0.94	0.8	-0.21	0.76	3.99
STI0032	$(GGA)_n$	V	128-143	4	3.47	0.89	0.73	-0.25	0.68	3.91
STI0033	$(AGG)_n$	VII	131-152	4	3.48	1	0.73	-0.4	0.68	3.14
STM0031	$(AC)_5$…$(AC)_3(GCAC)(AC)_2(GCAC)_2$	VII	186-206	4	2.92	0.78	0.68	-0.18	0.6	3.68
STM0037	$(TC)_5$ $(AC)_6$ AA$(AC)_7$ $(AT)_4$	XI	91-107	7	5.45	1	0.84	-0.22	0.81	6.19
STM1052	$(AT)_{14}$ GT$(AT)_4(GT)_6$	IX	226-244	5	2.82	0.56	0.66	0.14	0.56	4.24
STM1053	$(TA)_4$ $(ATC)_5$	III	187-191	3	1.74	0.56	0.44	-0.3	0.15	2.83
STM1064	$(TA)_{12}$.. $(TG)_4$ GT$(TG)_5$	II	207-212	3	1.18	0.17	0.16	-0.07	0.16	2.59
STM1104	$(TCT)_5$	VIII	183-197	6	4.02	0.89	0.77	-0.18	0.73	5.19
STM1106	$(ATT)_{13}$	X	169-213	5	3.6	0.5	0.74	0.31	0.7	4.14
STM5114	$(ACC)_7$	II	299-314	5	3.43	1	0.73	-0.41	0.67	2.1
STM5121	$(TGT)_5$	XII	301-310	4	1.99	0.44	0.51	0.11	0.32	3.14
STM5127	$(TCT)_5$	I	254-289	7	4.7	0.72	0.81	0.08	0.77	4.71
STPoAc58	$(TA)_{13}$	V	249-257	3	1.26	0.17	0.21	0.18	0.2	1.94
Overall mean				5	3.51	0.78	0.68	-0.17	0.62	4.12
SE				0.29	0.24	0.05	0.04	0.04	0.21	0.23

Na = No. of allele; Ne = No. effective allele; Ho = Observed heterozygosity; He = Unbiased expected heterozygosity (gene diversity); F = Fixation Index (inbreeding coefficient); PIC = Polymorphic information content; SE = standard error; bp = base pair; Ar= allelic richness;

A similarity matrix based on the proportion of shared SSR alleles was used to establish the level of relatedness between the various clones studied. Pair-wise estimates of similarity

ranged from 0.26 to 0.52 and the mean similarity among 18 clones was 0.35 (Table 5.3). Two clones, 396034.103 and 396031.108, were the closest related genotypes with the highest similarity index of 52%. The lowest similarity (26%) was observed between clones 395077.12 and 396029.25. As expected due to the heterozygous and heterogeneous nature of potato, genetic similarities between the clones were low indicating that the clones shared less number of alleles.

Table 5.3 Genetic distance estimates of 18 potato genotypes revealed by 23 SSR markers

Clones	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	0.00																	
2	0.33	0.00																
3	0.29	0.29	0.00															
4	0.39	0.33	0.29	0.00														
5	0.41	0.36	0.32	0.41	0.00													
6	0.34	0.32	0.36	0.46	0.34	0.00												
7	0.37	0.36	0.34	0.46	0.36	**0.52**	0.00											
8	0.27	0.33	0.37	0.34	0.33	0.28	0.29	0.00										
9	0.34	0.27	0.29	0.38	0.37	0.28	0.29	0.30	0.00									
10	0.32	0.33	0.31	0.35	0.30	0.38	0.44	0.33	0.27	0.00								
11	0.32	0.32	0.39	0.31	0.35	0.33	0.32	0.34	0.33	0.33	0.00							
12	0.34	0.28	0.28	0.32	0.29	0.27	0.29	0.29	0.27	0.29	0.36	0.00						
13	0.35	0.32	0.32	0.33	0.41	0.28	0.31	0.32	0.32	0.31	0.35	0.35	0.00					
14	0.47	0.33	0.34	0.42	0.44	0.40	0.41	0.32	0.39	0.32	0.34	0.33	0.36	0.00				
15	0.42	0.34	0.33	0.43	0.40	0.35	0.40	0.35	0.38	0.38	0.35	0.36	0.39	0.50	0.00			
16	0.32	0.33	0.29	0.33	0.32	0.24	0.29	0.34	0.32	0.27	**0.26**	0.27	0.29	0.32	0.33	0.00		
17	0.36	0.46	0.32	0.38	0.35	0.32	0.37	0.37	0.30	0.34	0.29	0.29	0.36	0.36	0.39	0.43	0.00	
18	0.32	0.39	0.29	0.36	0.38	0.30	0.30	0.31	0.30	0.32	0.32	0.36	0.32	0.36	0.35	0.38	0.44	0.00

1= CIP395015.6, 2= CIP395096.2, 3=CIP395109.34, 4= CIP395112.32, 5= CIP396004.263, 6= CIP396031.108, 7= CIP396034.103, 8 = CIP395011.2, 9 = CIP395017.14, 10= CIP395017.229, 11= CIP395077.12, 12= CIP396038.107, 13 = CIP392633.64, 14 = CIP393220.54, 15= CIP393371.58, 16= CIP396029.250, 17 = CIP396038.101, 18= CIP396038.105; G= genotypes.

The cluster analysis based on genetic dissimilarity using the neighbour-joining method in DARwin 5.0 classified the 18 potato genotypes into three main clusters (Figure 5.1). Cluster I consisted of seven genotypes: one clone (396029.25) was drought and late blight resistant, four clones were late blight resistant but drought sensitive (396031.108, 396034.103, 395096.2 and 396004.263) and two of them were late blight susceptible (392633.64 and 395015.6). Cluster II composed of the another seven clones: three drought tolerant but late blight susceptible clones (396038.107, 396038.101 and 395112.32) and four late blight resistant clones with various levels of drought tolerance (396038.105, 393220.54, Belete and 395077.12). Cluster III had four clones: two clones with moderate resistance both for drought and late blight (395017.229 and 395109.34), clone 395011.2 which is late blight resistance but drought sensitive and 395017.14, a clone susceptible for both drought and late blight disease.

The principal coordinate analysis (Figure 5.2) revealed three clustering patterns that existed among 18 potato clones. The first two principal coordinates explained 35.6% of the total genetic variance. In this clustering it was observed that clone 396038.101 was placed far from clones 395017.229 and 395017.14 in the first coordinate (X-axis). In the second coordinate (Y-axis) clones 395109.34 and 393220.54 were placed farthest from 395096.2, 396004.263 and 392633.64. All the clones characterized as drought sensitive (Table 5.1) located on the positive side of second coordinate (Y-axis) except clone 396029.25, which was the only tolerant clone in this group. In contrast, the negative side of the second coordinate was dominated by moderately tolerant to tolerant clones for drought including 396038.101, 396038.107, 395112.32, 395017.229, Belete and 395109.34. Late blight resistant clones, however, were distributed evenly over both the coordinates. Overall, results showed that the microsatellite markers used clearly distinguished all the eighteen potato genotypes.

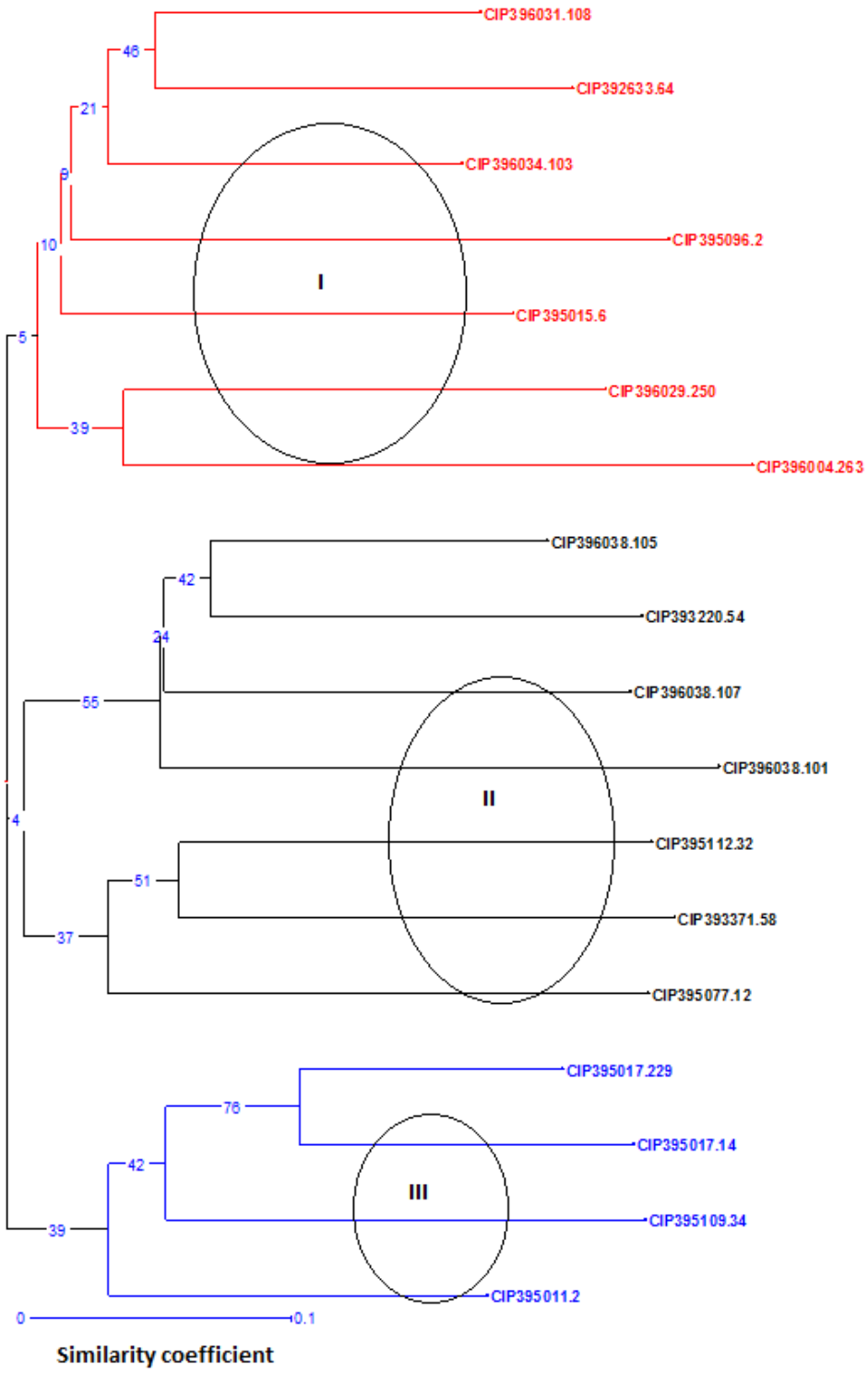

Figure 5.1 Neighbour-joining dendrograms showing genetic relationship among 18 potato clones using 23 SSR markers

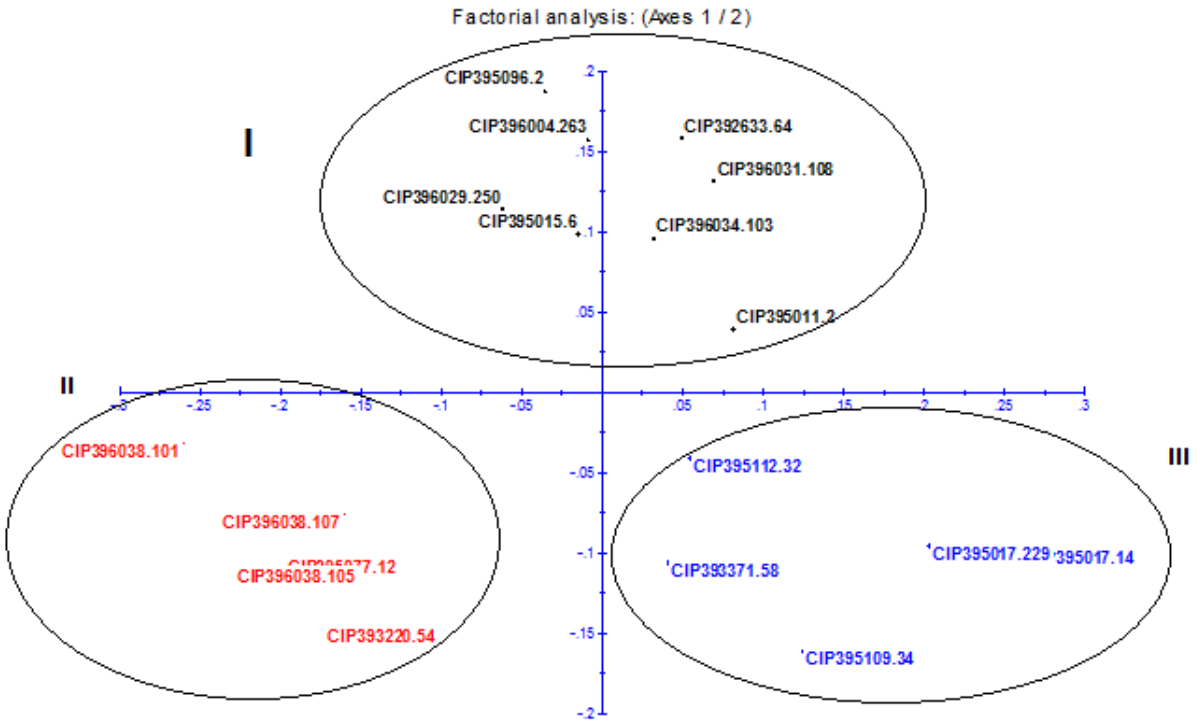

Figure 5.2 The first two principal coordinates of the principal co-ordinate analysis (PCoA) using SSR genetic dissimilarity matrix of 18 B3C2 potato clones.

5.4 Discussion

The present study examined the genetic diversity present among 18 potato clones from a B3C2 population using 23 SSR markers. The markers were previously selected and reported for their high polymorphism (Ghislain et al., 2004; Ghislain et al., 2009; Sharma and Nandineni, 2014). SSRs have the capacity to reveal ploidy status and their heterozygosity because of their co-dominant nature and they have the advantage of being highly polymorphic (Ghislain et al., 2004). Sharma and Nandineni (2014) and Milbourne et al. (1997) compared the DNA profiles obtained from SSRs with other molecular techniques among 47 potato varieties from India and found higher polymorphism and wider range of genetic similarity values in SSR markers than the other type of molecular markers. Besides, microsatellites are appropriate molecular tools to study the genetic distance of closely related germplasm sources (Ghislain et al., 2004). The selected clones are all tetraploids from the same species and from the same (B3C2) population, suggesting that the SSRs would be the best choice to distinguish the set of genotypes under the present study.

In the present study, 83% of the loci had PIC values of >0.5, which demonstrated the markers were very informative. The polymorphism values reported in this study ranged from 0.15 to 0.81 which is more or less similar to those reported by earlier studies. Ghislain et al. (2009) found PIC value of 0.25 to 0.88 using 51 SSR markers on 742 potato landraces with

different ploidy level. The observed fragment size of alleles for all loci was between 91 and 314 bp which is within the range of previously reported standard allele size (83-322 bp) (Ghislain et al., 2004; Ghislain et al., 2009). The allele size per locus coincides with the earlier studies except for the markers STM5127, STPoAc58, STM1106 and STG0016. The total number of alleles and number of alleles per locus observed in this study were low when compared to prior reports. For example, Ghislain et al. (2009) observed 137 alleles using 24 SSR markers (nearly all were used in the present study) on 742 potato genotypes with the range of 3 to 9 alleles per locus. Sharma and Nandineni (2014) found 139 alleles with allele per locus ranged from 6 to 11 using 17 SSR markers on 44 potato genotypes. The lower total number of alleles and number of allele per locus in the present study could be attributed to the smaller number of clones studied.

The observed heterozygosity within the potato genotypes was very high which is demonstrated by higher values for H_o than H_e for most of the SSR loci, except for STM1052, STM1106, STM5121, STM5127 and STPoAc58. Comparable results were obtained by Sharma and Nandineni (2014) on potatoes. The high observed heterozygosity confirms the predominantly outbreeding and tetraploid nature, and clonal propagation of the crop that preserve its heterozygosity. Constant monitoring of the B3 population to maintain sufficient genetic variation could also play a vital role for the observed heterozygosity (Landeo et al., 2001).

The cluster and principal coordinate analyses grouped the clones into three main clusters (Figures 1 and 2). It seems that the grouping mainly places potato genotypes in similarity clusters according to their tolerance to drought, although three of them were not phenotyped. There were seven drought tolerant and eight sensitive clones in the tested population, of which four tolerant clones (57%) were grouped together in cluster II and five of the susceptible clones (63%) were grouped in to cluster I. This could indicate the genetic bases of drought tolerance observed in this sets of clones were similar. The other explanation could be that the markers used in this study could be linked with genes conferring drought tolerance.

Conversely, late blight resistant genotypes were widely scattered in different clusters, suggesting a different genetic basis for resistance. The tested population had 12 late blight resistant and 6 susceptible clones. Both susceptible and resistant clones were dispersed over the three clusters and on principal coordinate plot, which reflected wider genetic variation within and among resistant and susceptible clones. Similar results have been reported by Pattanayak et al. (2002) who studied twenty-four tetraploid Indian cultivars that differed in late blight resistance using RAPD molecular markers. This result is in agreement

with wide range of variability and significant and preponderant additive gene action revealed in late blight resistance by crossing the subset of the clones in this study (Chapter 4). High genetic diversity implies the presence of a high amount of additive genetic variance, upon which progress in plant breeding depends (Carputo et al., 2013). The study also confirms stabilizing selection practiced in each cycle of in B3 population to maintain the genetic diversity for further breeding progress (Landeo et al., 2001). Kaushik et al. (2000) reported the presence of heterosis for late blight resistance. Thus, selecting cross combinations based on the genetic distance of late blight resistant clones could ensure further progress and provide an opportunity of identifying transgressive sergeants that can combat virulent pathogens of *Phyphthora infestance* and tolerate moisture stress.

In the light of this, clones 395017.229, 395109.34 from cluster III with drought tolerance and late blight resistance could be crossed with 396029.25 from cluster I with excellent resistance to late blight and drought but genetically distant from these two clones. Alternatively, the above clones could be crossed with drought tolerant clones 396038.107, 396038.101 and 395112.32 to complement their lack of late blight resistance. Hybridization of clones that showed close relationship in this study should be avoided to minimize genetic depression and reduced genetic variation in breeding materials.

5.5 Conclusions

Genetic diversity analysis is a useful tool to estimate genetic distance among genotypes and for an efficient choice of parents for breeding. Powerful molecular markers such as SSRs are neutral, stable and unaffected by environmental factors and are useful to study the genetic diversity among the various potato clones in a quick an accurate manner. The current study was therefore carried out to assess the pattern and extent of genetic diversity among the selected 18 potato tetraploid clones using 23 SSR markers to identify appropriate parents for crossing. The results showed that considerable genetic differentiation exists among the potato genotypes. The SSR genetic markers provided five distinct genetic groups. The following clones from different clusters were selected based on their late blight resistance and/or drought tolerance reaction: 396029.25 from cluster I, clone 396038.107, 396038.101 and 395112.32 from cluster II, and clones 395017.229 and 395109.34 from cluster III. Designing crosses based on genetic distance enable the breeders to exploit heterosis, and maintain genetic diversity for future improvement.

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An overview of the research findings

Introduction and objectives of the study

Potato is the third most widely grown crop worldwide serving as a staple food for more than one billion people. Ethiopia is amongest the top 10 potato producer in Africa. Seventy percent of the arable land of the country is situated in the highlands, which are potentially suitable for potato production. Potato is a strategy crop for food security due to its short growing season and higher yields per unit of land compared to other major cereal crops such as maize, rice and wheat. Potato production in Ethiopia is predominantly under rainfed condition and the productivity of the crop is highly vulnerable to seasonal rainfall variability which causes moisture stress (in dry season) or condition that favour the development of diseases (in wet season). Frequent drought and dry-spells during the growing seasons cause severe yield reduction, while humid weather enhances late blight disease development often leading to a complete crop failure. More than 95% of crop production in Ethiopia is under small-scale farming systems. Smallholder farmers grow potato on fragmented and small sized plots with limited use of irrigation water and chemical control of late blight disease. Therefore, genetic improvement of the crop through breeding for drought tolerance and late blight resistance could be the best strategy which is effective and affordable to smallholder farmers. This chapter, summarises the study objectives, highlights the main findings of each objective and their implications, and maps the way forward for future research.

The objectives of this study were:

- To select late blight resistant and high yielding potato clones under field conditions in the north-western parts of Ethiopia.
- To determine combining ability and gene action controlling late blight resistance, yield and yield components and to identify promising potato genotypes as potential parents in a breeding programme.
- To determine combining ability and gene action controlling yield, yield components and drought tolerance related traits among selected potato clones and to identify promising parents and crosses for cultivar development.
- To assess the level of genetic diversity among 18 selected potato clones using 23 simple sequence repeat (SSR) markers and to complement phenotypic selection for identification of suitable parents for breeding.

Research findings in brief

Response of potato clones to late blight disease, yield and yield related traits in north-western highlands of Ethiopia

Twenty four potato clones: 17 from B3C2 population and seven widely grown released and farmers' cultivars, were evaluated for late blight resistance and yield related traits at three locations and with two replication using random complete block design. The main findings of this study are presented below:

- Significant phenotypic variation was observed among the clones for late blight resistance and yield related traits under the disease pressure.
- Highest level of late blight resistance was found among B3C2 population sourced from the International Potato Centre.
- Five clones (396029.25, 395017.229, 396004.263 396034.103 and 395077.12) with high level of late blight resistance and yield performance were selected for further breeding and recommended for release after stability tests.

Combining ability of selected potato clones for resistance to late blight disease, yield and yield components

Eighteen F_1 families were generated from two sets of 12 parents using North Carolina Design II. The families were evaluated for late blight resistance and yield related traits in two locations using a randomized complete block design with two replications. Results showed that:

- The general combining ability effects (GCA) and specific combining ability effect (SCA) effects were significant for all the traits evaluated.
- The GCA effect accounted for 71% and between 53 to 80% of the genetic variation in the families for late blight resistance and yield related traits, respectively.
- Parental clones 396264.14 and 395109.34 showed good GCA effect for both late blight resistance and yield related traits, while clone 396004.263 was a good general combiner for late blight resistance.
- Crosses from 396004.263 x 395017.229 and 395096.2 x 396012.288 were selected for their significant SCA effect for both late blight resistance and yield related traits towards the desired direction.

- The genotypes from the cross of 395109.7 x 396264.14 were the best specific combiners for late blight resistance.

Combining ability of selected potato clones for drought tolerance and yield components

Thirty-two potato families derived from two sets of 16 parents using a North Carolina Design II together with 17 clones were field evaluated for yield and drought related traits in a 7 x 7 lattice design with two replications under irrigated and drought stress conditions. Results revealed that:

- There was significant variation among the genotypes tested in terms of all assessed traits.
- Significant GCA effects and SCA effects were found for yield and drought tolerance related traits and the GCA effects were more important than SCA effects for most of the traits measured.
- The following clones: 395112.32, 396034.103 and 396012.288 were found to be the best general combiners for yield and drought tolerance.
- Genotypes derived from the crosses of 395109.34 x 396041.102, 395096.2 x 396012.288, 395109.7 x 395017.14 and 396031.108 x 395017.14 had good SCA effects for tuber yield and drought tolerance.

Genetic diversity analysis of selected potato genotypes using SSR markers

Eighteen potato clones phenotyped for drought tolerance and late blight resistance were genotyped using 23 polymorphic SSR markers for an efficient choice of parents. Results showed that:

- A wide range of genetic diversity was observed among the tested genotypes.
- Neighbour-joining cluster analysis and principal coordinate analysis revealed the presence of three distinct genetic groups.
- Clones 396029.25 from cluster I, 396038.107, 396038.101 and 395112.32 from cluster II, and 395017.229 and 395109.34 from cluster III were selected as promising parents based on their desirable phenotype for drought tolerance, late blight resistance and their genetic distance.

Implications of the research findings for breeding potato for late blight resistance and drought tolerance

Results from the clonal evaluation demonstrated the presence of high level of late blight resistance among the B3C2 population. This population is developed by the CIP and is adapted to highland tropics and possess horizontal late blight resistance free of the major genes. Five clones were selected from this population with promising late blight resistance and high yields. These clones can be exploited in resistance breeding programs in the country. Also the clones are recommended for direct production after stability tests. Overall, the source population offered great opportunity for late blight resistance breeding in the Ethiopian highlands. However, genetic diversity studies of the pathogen should be considered in future to devise the most effective strategy for resistance breeding.

The present combining ability studies identified best parents and cross combinations with high yield and adequate late blight resistance. The selected clones could be effectively utilized in potato breeding to develop improved potato cultivars in Ethiopia. Additive genetic variance were found to be more important than non-additive variance in inheritance of resistance to late blight, implying the use of recurrent selection could provide better recombination and accumulation of desirable genes. Future studies should incorporate reciprocal crosses to rule out any possible maternal effects that may influence late blight resistance.

In the combining ability analysis for drought tolerance, additive genetic effects were found to be more important than non-additive variances in the genetic control of yield and drought tolerance related traits. This suggests that progress in increasing the level of drought tolerance can be achieved through breeding and recurrent selection. The selected parents and families are useful genetic resources to improve drought tolerance and yield of potato. The study demonstrated that there is no negative association between yield potential and drought tolerance, suggesting that drought tolerant cultivars are not necessarily associated with yield penalty under non-stressed condition.

Genetic diversity analysis using SSR markers provided three distinct genetic groups. Hybridization between the genetically distant clones could enable exploitation of hybrid vigour.

In summary, the present study identified genetically distant genotypes combining high yield, drought tolerance and adequate level of late blight resistance for developing improved potato cultivars in Ethiopia. The selected clones are important genetic resources to enhance potato

productivity in the highlands of Ethiopia or similar environments in sub-Saharan African countries.